PHOSPHATE NUTRITION OF PLANTS GROWN ON SALINE SOILS

by

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ABSTRACT

Experiments indicated that fertilizer phosphate had a much greater effect on the yield of cereal crops in saline areas than on well drained soils in the same field. The relationship between the increase in yield resulting from phosphate fertilizer and the level of salinity was curvilinear, increasing at low levels of salinity and decreasing at higher levels. The point of maximum response was variable, apparently depending on environmental factors. However, the degree of response (increase in yield divided by the check yield) increased continuously with increasing salinity.

The relationship between salinity and phosphate absorption by cereal plants was also found to be curvilinear, corresponding very closely to the yield increase curve except that it was more pronounced. This latter statement applies only to fertilized plants, presumably the supply of phosphate limited absorption in the absence of fertilizer, although even on the control plots phosphate absorption increased slightly with increasing salinity. At high levels of salinity plants growing in unfertilized soil showed negative phosphate gains. Barley plants grown for two weeks in phosphate free, saline solutions at 12° C, lost 26% of their original phosphate to the solution. No loss occurred at 18° C.

Metabolic studies indicated that respiration as measured by O_2 consumption and cytochrome oxidase activity were not specifically reduced in saline solutions, although the activity per plant decreased because of reduced growth in the saline media.

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INTRODUCTION

The saline depression is a characteristic landscape feature in sub-humid, semi-arid and arid regions, throughout the world. It is believed (40) that chemical weathering of igneous rocks is the original source of the majority of the soluble salts found on the earth's crust, with volcanic action contributing a significant portion of the chlorides and sulphates (53). However, these are extremely slow processes and in the majority of cases, the salts found in modern soils have originated from secondary mineral deposits, where they accumulated in past ages. In glaciated regions, saline materials were frequently intimately mixed with the parent material of the modern soil and salts have accumulated where evaporation of seepage or runoff water has exceeded the drainage rate.

The most widely accepted system of classification for saline soils is the one proposed in the U.S.D.A. Agricultural Handbook 60 (94). In this system, a saline soil is defined as a soil for which the specific conductivity of the saturation extract is more than four millimhos at 25° C. and the exchangeable sodium percentage is less than 15% of the total exchange capacity of the soil. Within this group the degree of salinity is defined by the electrical conductivity of a saturation extract of the soil.

The cause of unfavorable plant growth under saline conditions, has been investigated intensively, and a

number of comprehensive reviews of the experimental results have been published (3, 42). A number of specific hypotheses have been proposed to account for the deleterious effects of high salt concentration in the growth medium. The most important of these is the osmotic inhibition theory. Pfeffer (1877) is credited by Harris (40) with first recognizing the role of soluble salts as they influence the water economy of the plant. Since that time it has been shown that the growth of plants is highly dependent on the osmotic pressure of the growth medium (2, 32, 33, 43, 44, 76, 95).

In many cases however, specific salts or ions have been shown to have a greater effect on plant growth than iso-osmotic solutions of other soluble materials. It has therefore, been necessary to postulate specific toxic effects for individual ions.

The ions which most frequently accumulate in saline soils are: Na^{+}, Ca^{++}, Mg^{++}, Cl^{-}, $SO_4^{=}$, HCO_3^{-}, and NO_3^{-}. All of these ions have at one time or another, been reported toxic to one or more species of plants. In many instances the cause of the observed toxicity has been attributed to the effect of the ion on the ability of the plant to absorb an essential nutrient. Sodium, for example, has been reported toxic to a number of plants (3), the toxicity symptoms generally being associated with a Ca^{++} deficiency.

The imbalance of ions in the nutrient solution was stressed as a major cause of salt damage (10, 11, 36, 83, 84) prior to the general acceptance of the osmotic inhibition theory. However, demonstrations of this "antagonistic" action have been limited to the essential cations and have never been stressed in the case of anions. A number of authors (23, 34, 94) believe that the available evidence indicates that anion interference from saline substances is of minor importance and that decreased growth on saline media is not related in any appreciable degree to decreased availability of the essential anions.

In view of these reports, observations from fertilizer experiments which indicated a high degree of correlation between the level of salinity in the soil and response to phosphate fertilizers appeared to warrant further investigation. The investigations were conducted to determine if plant response to phosphate fertilizer is specifically related to soil salinity and if so, to investigate the possible causes of this relationship.

REVIEW OF LITERATURE

The saline soils encountered in Western Canada are usually calcareous. Chang and Jackson (17) have estimated that 95% of the phosphate contained in calcareous subsoil is in calcium forms. Consequently a study of the phosphate requirements of plants growing on these soils would be dependent on the chemical characteristics of the calcium phosphates.

McGeorge and Breazeale (69) concluded that in calcareous soils, calcium phosphate existed in a molecular carbonate-apatite $(Ca_3(PO_4)_2)_3.CaCO_3$. Eisenberger et al. (24) list the following calcium phosphates, dicalcium phosphate, tricalcium phosphate, hydroxyapatite, apatite, and tetracalcium phosphate. They state that the stable existence of tricalcium phosphate and tetracalcium phosphate is doubtful in the presence of water and that the existence of a unique stoichiometric hydroxyapatite is doubtful. They conclude that in the ternary system $CaO:P_2O_5:H_2O$ there exists a continuous series of solid solutions having an apatite lattice. Eisenberger et al. (24) state that a molecular apatite-carbonate has also been proposed as a constituent of bone, but Burns and Henderson (14) presented evidence which made this appear improbable.

Burd (13) concluded that in calcareous soils, phosphate existed as mineral aggregates rather than

molecular species and that the solubility of phosphate would depend on the calcium activity. Clark (18) presented evidence for the existence of hydroxyapatite in the form of reproducible solubility products, but stated that in the presence of CO_2 a reproducible solubility product could not be obtained.

The concept of a range of solid solutions containing varying proportions of calcium phosphate and carbonate appears to be widely accepted at present. Eisenberger et al. (24) pointed out that if this is true the composition of the product will largely be determined by the conditions under which precipitation occurs. Lindsay and Moreno (62) studied phosphate phase equilibria in calcareous soils and point out that under natural conditions equilibrium is not likely to be attained and that phosphate solubility would not correspond to any known solubility product.

Olsen (80), Cole et al. (20) and Shapiro and Fried (88) have reported on detailed studies of the adsorption of phosphate ions on the solid phases in the soil system. A high level of phosphate adsorption appears to occur on carbonate particles and precipitation reactions are relatively slow, Olsen et al. (82) demonstrated that calcareous materials in soils do not behave like calcite in their reactions with phosphate and attribute much of the difference to the colloidol nature of the soil carbonates and to their high surface area. Lehr and Brown (56)

believe that the comparatively slow adjustment to more basic forms of phosphate in the soil can contribute greatly to the availability of phosphate by reaction such as the following:

$$4(CaHPO_4.2H_2O) = Ca_4H(PO_4)_3 \cdot 3H_2O + H_3PO_4.5H_2O$$

Mattson et al. (a) as reported by Olsen (80) also stress the importance of the adsorbed phase in phosphate nutrition. Their studies dealt primarily with the aluminum phosphates and the amphoteric nature of the phosphate molecules is considered an important characteristic. On the acid side of the iso-electric point the ion atmosphere would be dominated by anions and on the basic side by cations. The extent to which this type of reaction might occur in calcareous soils is not known. Olsen (80) believes that precipitation reactions would remain predominant in the presence of calcium carbonate. Shapiro and Fried (88) illustrated that two phases of phosphate exist in the soil, one which is much more readily removed than the other by a leaching solution. This is presumed to correspond to adsorbed phosphate. Perkins (85) showed that the addition of phosphate to finely ground soil resulted in large increases in the cation exchange capacity of the soil. This again indicates the importance of the surface reactions of phosphate compounds.

(a) Mattson S. et al., Ann. Roy. Agr. Coll. Sweden. 18: 128-153 (Swedish) as reviewed by Olsen (80)

Recently a number of papers have been published in which the reactions which occur following the placement of granules of monocalcium phosphate in the soil have been studied. Lindsay and Stephenson (59, 60, 61), Brown and Lehr (12), Lehr et al. (57) and Bouldin and Sample (5) have all reported on studies of this type. The reaction sequence is summarized by Lindsay and Stephenson (59) as follows: "Water moved towards the fertilizer granule both by vapor and liquid transfer until a visible wet ring formed around the granule. The highly concentrated solution in the immediate vicinity of the granule expanded by capillary flow, dissolving iron, aluminium, and manganese, as it moved radially outwards. Calcium, aluminium and iron phosphates precipitated at the periphery of the wetting zone." In all cases it has been reported that hydrolysis occurred in the granule and a shell of dicalcium phosphate dihydrate remained at the original site. This shell contained from 20 to 30% of the original phosphate depending on the base status of the soil. Bouldin and Sample (5) measured the radial distance of movement from granules of monocalcium, monoammonium and diammonium phosphate. The maximum movement was less than 3 cm, with diammonium phosphate showing the greatest movement. All of these authors have reported that dicalcium phosphate dihydrate is the first calcium phosphate to precipitate and

that the conversions to more basic forms are extremely slow. Moreno et al. (73, 74) have published the results of studies on the solubility of dicalcium phosphate dihydrate and of octocalcium phosphate, (which they report as the second basic phosphate to precipitate from fertilizer phosphate applied to calcareous soils). The solubility product constants reported are in the range of 10^{-7} and 10^{-47} for the dicalcium and octocalcium salts respectively. They report that both of these compounds are unstable in aqueous systems but that hydrolysis reactions are so slow that equilibrium conditions can be assumed.

Due to the instability of the basic phosphates it has long been anticipated that salts would have a comparatively large effect on phosphate reactions in the soil. Wild (96), reports that Liebig postulated that neutral salts would increase the solubility of soil phosphates and that salts containing a common ion would decrease their solubility according to the then newly discovered "salt effect" and "common ion effect". However, in 1949 Wild (96) reviewed a large amount of literature concerning the effect of various salts on the solubility of soil phosphates and reported that there was as yet insufficient knowledge to explain the contradictory results.

The more recent literature is also somewhat contradictory. Bouldin and Sample (4) and Howe and Graham (47) have reported that the effect of salts on phosphate avail-

ability in soils is small and inconsistent. Starostka and Hill (89) compared the effect of a large number of saturated solutions of salts on the solubility of dicalcium phosphate in aqueous solution. In this test all salts containing anions which form insoluble calcium salts markedly increased the concentration of phosphate in the solution. Neutral and non-reactive salts, slightly increased the phosphate concentration, and all the calcium salts, and magnesium carbonate slightly decreased the concentration of phosphate in the solution. They report that similar results were obtained on soils. Lewis et al. (58) have reported similar results except that Na_2SO_4 was found to increase phosphate concentration at low rates of application and depress it at high rates. Lehr and Wesemael (55) reported that neutral salts depressed the solubility of phosphate in the soil with the cation having the greatest effect. Among the cations tested sodium containing salts had the greatest effect. Fine and Carson (31) reported that the addition of sodium sulphate or calcium sulphate to soils decreased the response to applied phosphorus.

The contradictions appearing in the literature make it apparent that the effect of salts on phosphate solubility in the soil is much more complex than in pure chemical systems. In general the soil solutions behave in a manner

similar to aqueous solutions, but the presence of the soil complex has a buffering effect on the reactions altering the order of magnitude to a lesser or greater extent depending on the properties of the test soil. Olsen et al. (82), present evidence of this concept by comparing the effect of sodium bicarbonate on the solubility of phosphorus in calcareous soils and of various calcium phosphates in the presence of calcite. Many of the contradictions might also be explained by the effect of dilution. Reitemeir (87) showed that on dilution, soluble calcium and magnesium replace exchangeable sodium and potassium and that the phosphate, chloride, and nitrate content of all soils increased on dilution. The increase in soluble phosphate may be due to changes in the cation status of the liquid phase. It is obvious that the chemistry of the phosphate ion in calcareous soils is only imperfectly understood and that quantitative prediction of the effect of salts is not possible. However, as Olsen (80) has pointed out the system could not be described completely even with perfect knowledge of the chemistry of the soil, since the effect of salts on the plant absorption mechanism must also be taken into consideration.

Modern concepts of the mechanisms involved in nutrient absorption by plants would lead one to expect that soluble salts might have a profound effect on the

proportions of the various ions absorbed and, consequently might cause nutrient deficiencies. Until comparatively recently nutrient absorption was considered to be a purely physical phenomenon and the transpiration stream was believed to be responsible for the translocation and absorption of nutrients by the plant. Hylmo (49) and Wright and Barton (97) have presented new evidence indicating that a small component of absorption is dependent on transpiration. The concept of "apparent free space" as advanced by Briggs and Robertson (8) and Butler (15), (which postulates a portion of plant tissue into which both solvent and solute can diffuse freely as opposed to the osmotic volume of tissue into which solvent alone can diffuse freely), has been used by Hylmo (49) as an explanation of the transpiration component of nutrient absorption. He postulates that a tenuous, but interconnected pathway of free space exists throughout the plant, through which the transpiration stream can transport a limited amount of the solution bathing the plant roots. If this hypothesis is correct a highly saline solution would affect mineral nutrition greatly, because it has been shown (33) that transpiration is reduced by increasing substrate concentration.

Since Hoagland and his collaborators (45) demonstrated that nutrient absorption is not a purely physical phenomenon, but is mediated by plant metabolism, a large amount of

research has been conducted aimed at elaborating the observed linkage between metabolic processes and nutrient absorption. Although the investigations of this problem have not resulted in the establishment of a universally accepted reaction mechanism, a number of component reactions of the absorption process have been described in detail and are well understood.

It has become evident that for purposes of study it is advantageous to divide the absorption process into two phases, a passive component, which results from purely physical factors and does not require the expenditure of energy by the plant, and an active component which is dependent upon direct energy expenditures by the plant. Elements entering the plant in the passive component do so with a favorable free energy gradient and the mechanisms are simple diffusion, exchange adsorption and possibly chelating or precipitation reactions. The term adsorption is usually applied to these mechanisms and adsorbed ions are freely exchangeable with the substrate, governed only by the relative bonding energies of the colloids involved. The active component is absorbed against a free energy gradient, it is non-exchangeable, selective and directly dependent on the release of energy by the plant. As pointed out by Briggs (9), this distinction is somewhat ambiguous since the identity of the ions concerned is not maintained and adsorbed nutrients are readily accumulated by active transport.

The importance of the passive component in the overall accumulatory mechanism of the plant has not been conclusively demonstrated. Lundegardh (65, 66) believes that the adsorption phase is an integral part of the process serving as the source of ions for accumulation. Steward and Sutcliffe (91) are inclined to agree with this view but Epstein (28) and his co-workers do not believe that a causal linkage exists. Regardless of whether the so-called first or adsorption phase is essential to active transport or not it is undoubtedly an important characteristic of plant roots.

Mehlich and Drake (70) credit Devaux (1916) with first demonstrating the capacity of plant roots to adsorb cations by an exchange process. Jenny and Overstreet (52) studied this phenomenon and the interchange of cations between soil and plant colloids, elaborating what has become known as the theory of "contact feeding". Huffaker and Wallace (48) and Drake and Steckel (22) have studied cation exchange by plant roots, attempting to show that this property of the roots could effectively reduce the activity of cations in the soil solution and consequently increase the solubility of the calcium phosphates. Noggle and Fried (78) studied the effect of the cation exchange capacity of plant roots on phosphate adsorption and found a high degree of correlation.

While the mechanism of active transport has not yet been settled the studies conducted have contributed much towards an appreciation of the factors influencing the process and to an understanding of the kinetics of the component reactions. Active transport is highly dependent on temperature (15, 51) having a temperature coefficient characteristic of chemical reactions, it is blocked by respiratory inhibitors (38, 46), and displays reaction kinetics similar to enzymic reactions (15, 28, 38, 46). The most widely accepted hypothesis, concerning the mechanism involved in active transport (28, 38, 46, 78, 91) requires the existence of metabolically produced carrier molecules, which adsorb ions on the substrate side of the cellular diffusion barriers and release them within the osmotic volume. The kinetics of the system suggest that a number of different carriers exist which are partially specific with respect to the ions transported.

Ions with similar properties behave as metabolic analogs and compete for identical sites on the carriers while dissimilar ions show non-competitive inhibition, in a manner similar to non-competitive inhibitors of enzyme reactions (75).

Stenlid (90) has listed a number of factors concerning the phosphate ion which distinguish it from other ions in respect to absorption by plants.

"1. Phosphate is present as two ionic species (within the biological pH range) which are probably absorbed by different carriers.

2. The initial concentration of phosphate in the plant is high.

3. Phosphate uptake is slow and losses occur from roots. These losses can be counteracted by the addition of sugar to the substrate.

4. Absorption of phosphate is stimulated by sugars.

5. Divalent ions particularly Mg^{++} stimulate the absorption of phosphate.

6. Phosphate absorption is more readily inhibited by dinitrophenol than other ions.

7. Phosphate is rapidly incorporated into organic molecules within the plant."

Olsen et al. (81) calculate that the mole ratio of $H_2PO_4^-$ to $HPO_4^=$ is 1 at pH = 7.2. At pH 5 the solution is 99.3% $H_2PO_4^-$ and at pH 9 it is 98.4% $HPO_4^=$. Hopkins (46) studied the kinetics of phosphate uptake and postulated that the two ionic species of orthophosphate were absorbed by different carriers and did not compete with each other. Hagen et al. (38) reported evidence confirming this view and by the use of respiratory inhibitors indicated the respiratory reactions related to absorption of the two ionic species. Noggle and Fried (78) using similar methods

studied the relative concentrations of the carriers in a number of plant species and found that uptake rates were correlated with site concentrations.

Nye and Foster (79) demonstrated a net loss of phosphate to substrates low in phosphate. Fedorovskii (30), Emmert (25) and Von Michael and Marschner (71) have demonstrated the same phenomenon under varying conditions.

Loughman and Scott-Russell (63) demonstrated that phosphate is incorporated into esters in less than one minute after entry into the plant. Nucleotides were the first products formed and the phosphate status of the plant affected the pattern of esterfication. Jackson and Hagen (50) demonstrated that uridine diphosphate glucose (UDPG) is one of the first products formed and postulated that phosphate absorption is coupled to oxidative phosphorylation.

Studies designed to measure the competitive effects of various ions on phosphate absorption conducted by Lundegardh (67), Butler (15), Olsen (80), Eaton (23), Gauch and Wadleigh (34), and Leggett and Epstein (54), have shown that phosphate absorption is not competitively inhibited by sulphate, chloride or nitrate, but the bicarbonate and hydroxyl ions both compete for phosphate carriers. Gausman and Awan (35) demonstrated that chloride increased the rate of phosphate absorption into potato tubers at low concentra-

tions but inhibited it at higher concentrations. The first papers cited all dealt with the competitive effect of the various ions at very low concentration levels, with the exception of the papers by Gauch and Wadleigh who were working with comparatively concentrated solutions. No references have been found pertaining to the effect of non-competitive inhibition of phosphate uptake by other ions in solutions approximating the concentrations found in saline soils.

MATERIALS AND METHODS

Field Studies

Generalized description of soils All experiments were conducted on members of the Souris soil association similar to those described below.

Orthic Black.

Horizon (a)	Depth	Description
Ah	0 - 8"	very dark grey sandy loam; single grained to granular; soft; pH 7.5. The color gradually grades to a very dark grey brown transition layer.
AB	8 - 14"	very dark grey brown sandy loam; massive; pH 7.7.
Bm	14 - 22"	very dark brown loamy sand; large blocky to poorly developed columnar structure; pH 7.8.
Ck	22 - 30"	very light grey loamy sand; massive structure pH 7.9.
C	30" +	Stratified shaly and iron stained sand.

(a) National Soil Survey Committee of Canada. Report of the meeting; Guelph, Canada, February, 1960.

Saline Black.

Horizon	Depth	Description
Ah	0 - 8"	very dark grey sandy loam; granular; soft; 3 to 10 millimhos conductivity; gypsum concretions; pH 7.8.
AB	8 - 12"	light grey brown sandy loam; structureless material; 2 - 8 millimhos conductivity; pH 8.0.
Ck	12 - 18"	light grey loam; structureless; 1 - 6 millimhos conductivity; very wet; pH 8.1.
C	18" +	mottled yellow and bluish grey clay loam; 1 - 4 millimhos conductivity; pH 8.0.

Plot techniques. Seeding was accomplished by means of a power driven, four bank, V- belt seeder, using double disk seed openers. The fertilizer materials were placed on the belt together with the seed, and the belts were calibrated to seed rows twenty-four feet in length. Four rows, spaced nine inches apart constituted a single treatment, and one rod was harvested from the center two rows of each plot for yield estimations.

Laboratory and Green house Studies

Method of seed preparation. The following methods were used while preparing seed for the laboratory and greenhouse experiments reported in Sections II to VI.

A large sample of barley (Hordeum vulgare, var. Parkland) was passed through an eight-inch cleaning mill a sufficient number of times to give a uniform sample of approximately thirty pounds of grain, which was used in all future experiments. Each new seed lot was drawn from this supply, shaken seven times in sterile distilled water and spread on a sheet of foam plastic saturated with freshly aerated sterile tap water in a glass tray. The seeds were covered with moist cheese cloth and left in a dark cupboard in the laboratory for twenty-four hours prior to seeding.

Potting and harvesting methods. The following methods were used in the greenhouse experiment reported in Section II.

Thirty pounds of greenhouse grade vermiculite were mixed with thirty pounds of calcareous sand (obtained from the zone of carbonate accumulation in a local sand pit), after slight moistening to prevent mechanical separation. Samples of this mixture were taken to determine moisture holding capacity and inorganic carbon content. The moisture holding capacity was determined by allowing the saturated material to come to equilibrium over dry material, under a polyethylene cover. The sand-vermiculite mixture was then divided into lots sufficiently large to fill four, six-inch diameter plastic

pots. The different salts were added to sufficient water to wet the potting mixture to the moisture holding capacity. Following the addition of the salts and water equal weights of the potting mixture were added to each pot.

Harvesting was accomplished by removing the entire root medium from each pot and shaking the loose sand and vermiculite from the root mat. The roots were washed in running water over a 2 mm sieve until clean. The plants were then pressed firmly between paper toweling to remove excess water and weighed. They were weighed again after drying at 60° C. and ground for analytical purposes.

Growth chamber. A double-walled insulated cabinet, 8 feet long, 3 feet high and 2 feet deep was constructed. The cabinet had a glass top and four hinged doors at 2 foot intervals along the front. A hardboard shelf, drilled to accomodate forty-eight culture tubes was placed in the cabinet. This shelf divided the cabinet into an upper and a lower section of equal size. The culture tubes were suspended in the holes in the shelf so that one half inch of the tube protruded above the shelf and seven and one half inches protruded below the shelf. The inside portion of the chamber above the shelf was painted white and the lower half painted black to reduce reflected light.

Light was supplied by means of two eight-foot fluorescent tubes and four 500-watt incandescent bulbs situated above the cabinet. A white reflector was situated above

the lights which provided a total light intensity of 200 foot candles at the plant level.

The temperature was controlled in the cabinet by means of a thermostatically controlled "arctic type" cooler which was arranged to deliver air to a vent situated in the bottom of the cabinet. The air in the cabinet was nearly saturated at all times since cooling was accomplished by evaporation of water.

The culture tubes were prepared by cutting two-inch diameter glass tubing into eight-inch sections and inserting a two-hole rubber stopper in each. A capillary tube was placed in one hole of each stopper and connected by rubber tubing to a compressed air line. Air was bubbled continuously through each culture tube during the growth period. A short piece of $\frac{1}{4}$ inch diameter glass tubing was inserted in the other hole of each rubber stopper to facilitate solution changes. Six of these outlets were connected to a glass manifold by means of rubber tubing. The culture tubes were connected to the glass manifolds at random. The forty-eight culture tubes were therefore divided into eight groups of six tubes. The six tubes in each group were drained and filled simultaneously by means of the manifolds. An experimental unit consisted of all of the plants grown in the six tubes in each group. A photograph of one section of the cabinet is shown in Figure 1.

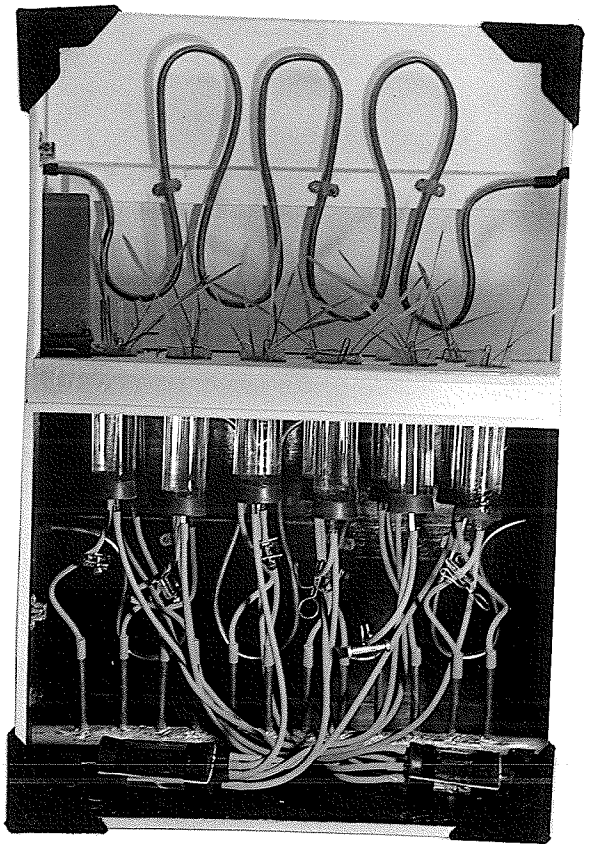

Figure 1. Barley plants growing in culture tubes in one section of growth chamber.

Nutrient solutions. Phosphate-free Hoagland's solution composed as follows, was used as a standard solution

Hoagland's Solution

0.005 M $Ca(NO_3)_2$

0.002 M $MgSO_4$

0.005 M KNO_3

This solution is referred to as 1 x Hoagland's - In some experiments the concentration was increased daily by multiplying these concentrations by 2, 3, 4, etc. The highest concentration prepared was 8 x Hoagland's used after eight days of growth.

The saline solutions were prepared using the salts commonly found in saline soils in the area, attempting to maintain the relative concentration similar to the normal salt distribution in saline soils of increasing salinity. The composition of the various saline solutions in moles per liter was as follows:

	SALINE SOLUTION NUMBER						
	1	2	3	4	5	6	7
$MgSO_4$	0.0037	0.0100	0.0200	0.0300	0.0400	0.0500	0.0590
Na_2SO_4	0.0012	0.0050	0.0100	0.0150	0.0198	0.0250	0.0294
NaCl	0.0015	0.0075	0.0150	0.0220	0.0278	0.0350	0.0433
$CaCl_2$	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003
KNO_3	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025

The seedlings were started in 1 x Hoagland's solution and the concentration increased by changing the solution daily, starting with saline solution #1 and using successively numbered solutions. Number seven solution was used on the eighth and succeeding days.

The specific conductivity of selected solutions was as follows:

Solution	Specific Conductivity Millimhos / cm
1 x Hoagland	2.08
8 x "	10.00
#1 Saline	0.98
#7 Saline	9.26

Analytical methods

Phosphate determinations (soil samples). Two methods were used to determine the amount of "available" soil phosphate. In the first method 0.5 molar sodium bicarbonate was used as an extracting solution, using a 1 to 20 (soil to solution) extraction ratio, as described by Olsen et al. (81). The ammonium fluoride-hydrochloric acid (0.03 N NH_4F and 0.025 N HCl) extractant described by Bray (7) was also used. The phosphate content of the filtrates from both extractants was determined by adding ammonium molybdate solution and developing the color of the reduced phosphomolybdate complex with stannous chloride solution. The

optical density of the solutions was determined in a Klett-Summerson colorimeter with a red filter and the readings compared to the values obtained with standard phosphate solutions.

Phosphate determinations (plant samples).

Total plant phosphate. was determined by the following procedure adapted from a procedure described by Toth et al. (92).

Reagents

Ammonium molybdate reagent. Twenty-five grams of reagent grade ammonium molybdate was dissolved in 1 liter of 10 N H_2SO_4.

Stannous chloride stock solution. Ten grams of stannous chloride dihydrate was dissolved in 25 ml of concentrated HCl and stored in a dark bottle and renewed every month.

Stannous chloride working solution. A 0.75 ml aliquot of stock solution was diluted to 250 ml with distilled water. This solution was replaced daily.

Standard phosphate solution. A solution containing 50 ppm P. was prepared by dissolving 0.2154 g KH_2PO_4 in distilled water in a liter volumetric flask and making to volume.

Procedure. A 2 g sample of oven dry plant material was weighed into a 300 ml Kjeldahl flask, 10 ml concentrated HNO_3 was added and the mixture was heated gently in a fume hood. When the plant material was thoroughly charred, the flask was cooled and 5 ml $HClO_4$ added. The mixture was

then boiled until clear and until dense white fumes appeared. The flask was cooled and 25 ml of distilled water added and this was boiled until about 5 ml of solution remained in the flask. The solution was cooled and filtered with repeated washing with hot water. The filtrate was diluted to 100 ml when cool. A 1.0 ml aliquot was pipetted into a 50 ml volumetric flask and diluted to about 25 ml. Three milliliters of ammonium molybdate solution was added and diluted to about 40 ml. Four milliliters of dilute stannous chloride solution was added and mixed by shaking and then diluted to 50 ml. After 15 minutes the optical density was measured with a photoelectric colorimeter against a reagent blank. The procedure was calibrated by the use of standard phosphate solutions in the range 0.2 to 4.0 ppm P.

Extraction of phosphate fractions. The procedure was adapted from the methods described by Pons et al. (86) and McCance and Widdowson (68).

Ether and benzene-alcohol soluble phosphate (lipid phosphate). Six grams of ground plant material was placed in a soxhlet extraction thimble and extracted for eight hours at low heat with diethyl ether. The ether was then removed by low temperature distillation and the material extracted for a further eight-hour period with a benzene-alcohol mixture (32.4 g of benzene plus 67.6 g ethanol). After removal of the second solvent the extract was digested

with nitric and perchloric acids and the phosphate content determined on an aliquot by the colorimeter procedure described previously.

Total phosphate. A 1 g sample of the fat free material was digested and the quantity of total phosphate determined as described above. The sum of the lipid phosphate and the total phosphate determined following fat extraction constituted the total plant phosphate.

Phytin phosphate. A second 1 g sample of the fat free material was shaken for two hours in 100 ml of 2% HCl (48.5 ml con. HCl + 100 g $Na_2SO_4.10H_2O$ diluted to 1 liter). After shaking, the plant debris was filtered and a 50 ml aliquot of the clear filtrate was transferred to a 100 ml beaker. One drop of phenolphthalein indicator and 2 ml of 5 N NaOH was added and the solution adjusted with 1 N NaOH and 1 N HCl until just colorless. Five milliliters of ferric chloride solution (15 g $FeCl_3.6H_2O$ in 500 ml of 1 N HCl) was added to the solution after heating on a steam bath and the precipitate allowed to cool after a 20 minute digestion period. The solution was then filtered and the precipitate and beaker washed with four washings of 0.6% HCl (14.5 ml. con. HCl + 100 g $Na_2SO_4.10H_2O$ to 1 liter).

The precipitate and paper were returned to the beaker and broken up in 5 ml of hot water. Two milliliters of 1 N NaOH was added and the solution was heated for 15 minutes on a steam bath.

The filter paper and precipitated ferric hydroxide were filtered and the filtrate evaporated in a Kjeldahl flask and digested with nitric and perchloric acids as above. Phosphate concentration was determined by the colorimetric procedure previously described and expressed as a percentage of the original dry weight.

Total acid soluble and inorganic phosphate. A 2 g sample of the fat free material was shaken for five minutes in 25 ml of cold 0.2 N HCl in a pre-chilled Erlenmeyer flask. The solution was then filtered through a Gooch crucible under suction into a 50 ml volumetric flask and made to volume by washing with ice cold water.

An aliquot (2 ml) of the filtrate was used to determine inorganic phosphate content immediately and a 25 ml aliquot was transferred to a Kjeldahl flask, evaporated to dryness and digested with nitric and perchloric acid. The phosphate content of the digest was then determined by the method previously described and expressed as % P on the basis of the original dry weight.

Protein and ester phosphates. The difference between the total phosphate in the fat free material and the total acid soluble phosphate was called protein phosphate.

The difference between the total acid soluble phosphate and the sum of the inorganic and phytin phosphate was designated as ester phosphate.

Soil pH and salinity measurements. The pH determinations were made with a Beckman model H-2 pH meter equipped with a glass electrode using the saturated paste method (94).

The degree of salinity in soil samples was estimated by measuring the specific conductivity of saturation soil extracts as described in U.S.D.A. Handbook 60 (94).

Ash content of plant material. A 2 g sample of ground plant material was ignited slowly in a tared 50 ml porcelain crucible to a temperature of 550° C. in a muffle furnace. The crucible was transferred to a desiccator and weighed when cool.

Inorganic carbon content of the soil was determined by digesting in dilute HCl and capturing the evolved CO_2 in standard base, according to the method described in the Methods of Analyses of the Association of Official Agricultural Chemists. (1)

The apparent density or volume weight of the soil samples was determined by measuring the oven dry weight of samples collected in a three inch Uhland core sampler.

Mechanical analyses of the soil samples were conducted using the hydrometer method as described by Bouyoucos (6).

Oxygen uptake by Plant tissue. Measurements were made by the direct method of Warburg as described by Umbreit et al. (93).

The roots were excised from the plants and cut into 1 cm sections by means of a multiple-razor-blade knife, and

placed in a beaker containing aerated solution identical to the rearing solution. After mixing, random samples of approximately 0.5 g fresh weight were taken from each solution, blotted dry, weighed and transferred to Warburg flasks containing 10 ml of the rearing solution. Ten percent potassium hydroxide was added to the center well and the flasks were placed on the manometers in a constant temperature bath at 16° C. (the temperature at which the plants were grown). After allowing one half hour for equilibration, the stopcocks were closed and the changes in pressure were recorded at ten minute intervals over a one hour period. After correcting for changes in atmospheric pressure, the data were plotted and the mean oxygen uptake values were computed from the best linear relationship, using the known flask constants.

Radioactive assay for phosphate absorption. Adapted from a procedure described by Butler (16).

The roots of two-week-old plants were excised and cut into 1 cm segments, by means of a multiple-razor-blade knife. The root segments were thoroughly mixed in a beaker containing freshly aerated solution of the same composition as the rearing solution. After mixing, 50 root segments were counted from each batch of roots and transferred to a 50 ml beaker containing 25 ml of the rearing solution.

In the meantime fresh culture tubes were prepared in the growth chamber containing 125 ml of one of the following solutions.

1. 1 x Hoagland's solution 10^{-6} molar with respect to KH_2PO_4.
2. " " " 10^{-5} " " " " "
3. " " " 10^{-4} " " " " "
4. #7 Saline solution 10^{-6} " " " " "
5. " " " 10^{-5} " " " " "
6. " " " 10^{-4} " " " " "

One milliliter of a solution containing radioactive phosphate (one micro Curie per 100 ml) as H_3PO_4 in dilute HCl, was pipetted into each culture tube. The beakers containing the root segments were emptied into the appropriate culture tubes at three minute intervals, thoroughly mixed and a 0.3 ml sample from each tube transferred to a stainless steel planchette. These planchettes were used to measure the specific activity of the original solutions when samples of the digested root segments were being counted.

Following a three-hour absorption period each solution was decanted through a nylon filter ring and the roots retained on the filter. The roots were washed three times with solutions similar to the absorption solutions but lacking radioactive phosphorus. The filter rings were then placed in centrifuge tubes and centrifuged at 1000 x g for 3 minutes to remove excess liquid. The root segments were transferred to tared crucibles and weighed, oven dried and reweighed. The roots were digested in the presence of magnesium acetate at 500° C. in a muffle furnace, the residue was taken up in

dilute hydrochloric acid and made to 10 ml. Radioactive assays were made by drying 0.3 ml aliquots of the digest on planchettes at very low heat and counting at constant time under an end window Geiger Mueller tube with a Philips model 4031 scaler. The amount of phosphate absorbed per plant was estimated from the known weight of roots per plant and from the specific activity measurements made on the original absorption solutions.

Determination of cytochrome oxidase activity. Adapted from a procedure described by Cooperstein and Lazarow (21).

Roots from two-week-old plants were excised into 50 ml beakers containing 10 ml of pre-chilled solution identical to the rearing solution. These were weighed and the fresh weight of the roots calculated from the difference in weight of the previously tared beakers. The beakers were left in a cold room (4° C.) for two hours to ensure complete chilling of the roots.

After two hours the roots were transferred to a cold mortar containing a small amount of acid washed silica sand and ground with approximately 5 ml of 0.5 M sucrose solution. The root debris was then pressed through a layer of cheesecloth into 12 ml centrifuge tubes. The filter and mortar were washed successively with 2 ml aliquots of 0.5 M sucrose until the centrifuge tubes were nearly full.

The tubes were centrifuged for 15 minutes in a refrigerated centrifuge at 1000 x g. The supernatant was decanted into a 25 ml centrifuge tube in an ice bath and the cell debris

resuspended in 5 ml of 0.5 M sucrose and recentrifuged and the supernatant again added to the 25 ml centrifuge tube. This procedure was repeated twice and finally the solids were discarded. The supernatant solutions collected in the 25 ml centrifuge tubes were centrifuged for 20 minutes at 20,000 x g. The supernatant was decanted and the particulate fraction resuspended in 10 ml of cold 0.5 M sucrose. The suspension was again centrifuged at 20,000 x g for 20 minutes and the supernatant discarded. The particulate fraction was then resuspended in sufficient cold 0.5 M sucrose to make the final suspension 0.14 g fresh weight per ml. The suspension was stored in an ice bath until activity measurements were completed on the same day.

Cytochrome oxidase activity of the suspensions was determined (21) by measuring the change in optical density of a solution of reduced cytochrome C at a wave length of 550 mu in a spectrophotometer, after addition of an aliquot of the particulate suspension.

Cytochrome C was obtained in the oxidized form from Sigma Chemical Co. Ltd., St. Louis, Mo. and was reduced with sodium hydrosulphite. The excess hydrosulphite was removed by bubbling oxygen through the solution and filtering. The cytochrome C was checked for auto-oxidation and found to be stable for the period of time used. Following activity measurements total nitrogen was determined on samples of the suspension by the micro-Kjeldahl procedure (1).

Activity measurements were carried out using the following reaction mixture in cuvettes of 1 cm light path.

1. 0.3 ml reduced cytochrome C (4.7 mg/ml).
2. 2.5 ml phosphate buffer pH 7.0 in 0.5 M sucrose.
3. 0.2 ml particulate suspension (0.14 g fresh wt. roots per ml of suspension).

Determination of apparent free space. Adapted from the procedure described by Butler (15).

The roots of plants growing in culture tubes were immersed in aerated nutrient solution (0.02 M with respect to mannitol designated solution B below) for 4 hours prior to harvesting. Following this adsorption period, the roots from 10 plants were excised into cheesecloth bags and centrifuged for 1 minute at 750 x g to remove excess solution, the roots were then rapidly weighed and transferred to 50 ml beakers containing 20 ml aerated, mannitol free, nutrient solution, designated as diffusion solution A below. The roots were gently agitated in the solution for 2 hours and filtered. The apparent free space of the roots was determined by measuring the quantity of 0.03 M mannitol in an aliquot of the filtrate and thus calculating the volume of the original solution transferred by the roots. The volume of the original solution transferred by the roots was divided by the fresh weight of the roots and multiplied by 100 to give % apparent free space.

Mannitol determinations. To a 5 ml aliquot of the diffusion solution A, and to a 5 ml aliquot of a 1/100 dilution of the original nutrient solution B, 5 ml KIO_4 solution (0.6 g KIO_4 in 20 ml H_2SO_4, diluted to 1 liter) was added. The solution was heated on a steam bath for 15 minutes and transferred to an ice bath. When cool the reaction vessel was washed with 1 ml of KI solution (12.5 g KI, 25 g $ZnSO_4$ and 125 g NaCl in 500 ml H_2O). This was titrated with 0.01 M $Na_2S_2O_3$ until pale yellow and 1 drop of starch solution (5 g soluble starch in 100 ml H_2O) was added and the titration completed. A reagent blank was titrated using an aliquot of mannitol free nutrient solution. This is designated as titre C in the calculations.

Calculations

$$\frac{20\ (\text{titre C} - \text{titre A})}{(\text{titre C} - \text{titre B}) \times \text{Fresh weight roots}} = \%\ \text{apparent free space.}$$

EXPERIMENTAL RESULTS

I. Field Studies. Comparisons of the effect of salinity on phosphorus absorption and yield of cereal crops.

A. A comparison of saline and non-saline soils in the same soil association.

During the 1958 and 1959 growing seasons fertilizer experiments were conducted on representative sites, selected on the orthic black and the saline black soils as described in Materials and Methods. These tests were conducted on summerfallow land, with both soils represented in a single field. In 1958, the plots were arranged in a non-repeated simple lattice design, and the yield data analysed according to the method of Cochran and Cox (19). Each soil type was represented by a single site. In 1959, the plots were arranged in a split-plot design with a total of six replicates. Two replicates per soil type, were located in each of three different fields. Spring wheat (Triticum vulgare, variety - Selkirk) was used as a test crop.

In 1958, the treatments consisted of three fertilizer formulations (11-48-0, 15-39-0, and 23-23-0) each of which was applied at five rates calculated to provide 10, 20, 30, 40 and 50 pounds P_2O_5 per acre. A non-fertilized control constituted the sixteenth treatment. In 1959, two rates of nitrogen application (15 and 40 pounds N per acre) and five rates of phosphate, (0, 10, 20, 40 and 60 pounds P_2O_5 per

acre) were applied. Ammonium phosphate (11-48-0 fertilizer as supplied by Consolidated Mining and Smelting Co.) was used as a source of phosphate in all cases and ammonium nitrate fertilizer (33.5-0-0) was used as a source of nitrogen.

In 1959, plant samples were collected at the late flowering stage to determine the quantity of phosphorus absorbed by plants grown with increasing quantities of fertilizer phosphate. Samples were collected from all sites and replicates, but only from those plots which had received 40 pounds N per acre. Sampling was accomplished by clipping, at ground level, six randomly selected one-foot lengths from the outer row of each plot. The yield of dry matter and of phosphorus and the percentage phosphorus were determined on these samples.

Soil samples were collected at all sites just prior to seeding. Sampling was accomplished by taking eight randomly distributed samples, representing the 0" - 4" soil depth, thoroughly mixing and drawing a sub-sample for analytical purposes (Table 4). The last item in Table 4 illustrates the degree of yield response to phosphate on each soil type (control yield as percentage of yield from the 40 lb. P_2O_5 treatment).

Analysis of variance indicated that no differences in yields could be attributed to rates of nitrogen application and that there were no interactions between rates of nitrogen and phosphate. Consequently, the data presented in Tables 1

and 2 are the mean of nitrogen rates. In Table 1 the yield data comparing the two sites is calculated on the basis of control yield equals 100 bushels per acre. The 1958 data are the mean of two replicates and three fertilizer formations while the 1959 data are the mean of six replicates and two nitrogen rates.

In Table 2, part A, the analytical data from July 9th sampling date are presented as the mean of two replicates. In part B, the yields of grain from the 1959 experiment are presented using units of cwt per acre. Each entry is the mean of two replicates and two rates of nitrogen application.

These experiments indicate that phosphate fertilizers have a much greater effect on the yield of wheat on the saline soil tested than on the non-saline soil (Table 1). The yield of dry matter of plants grown on saline soil (Table 2) is highly dependent on the amount of phosphate supplied. On the non-saline soils there is no consistent relationship between the effect of phosphate treatments on the yield of plant phosphorus on July 9th (Table 2 (iii)) and the effect of phosphate treatments on the yield of grain (Table 2B). Whereas on the saline soils, there is very good agreement between these two measures of the fertilizer effect.

These results suggest that the difference between the two soil types is due to the lack of available phosphate in the saline soil. However, since the percentage phosphorus

in the plants grown on two of the saline sites (Table 2 (ii)) is similar to that in the plants grown on the non-saline sites, it could be argued that the difference between the two soils is due to the inability of the plants to absorb fertilizer phosphate from the non-saline soil.

The significant interaction between phosphate rate and soil type (Table 3) applies only to the soils tested because soil types and fields were considered as fixed variates for the purposes of this analysis of variance. An extensive testing program would be required to establish the generality of this relationship between soil salinity and the effectiveness of phosphate fertilizer.

There appears to be no correlation between response to phosphate and $NaHCO_3$ extractable phosphate (Table 4). The conductivity of a saturation extract of the soil is the only item in Table 4 which appears to be correlated with the response to phosphate fertilizer. However, since there are numerous other factors which vary between the two soils, such as moisture supply and soil temperature, these observations are not conclusive evidence of a direct relationship between soil salinity and the availability of soil phosphate.

Table 1. A comparison of the effect of varying rates of phosphate on the yield of wheat grown on a saline and an orthic black soil in the same drainage basin.

Treatment lbs P_2O_5/A	1958		1959	
	Orthic	Saline	Orthic	Saline
	% Yield		% Yield	
0	100.0	100.0	100	100
10	113.4	132.0	111.1	109.1
20	117.4	142.6	113.6	128.7
30	119.8	153.8		
40	123.0	152.1	116.4	143.6
50	119.1	152.9		
60			122.0	172.1
Std. error	10.6	14.5	5.7	
Actual control Yield bu/A	17.8	29.0	23.4	16.3

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Table 2. A Comparison of the effect of varying rates of phosphate on the phosphorus content and the yield of wheat grown on saline and non-saline soil.

A. Sample collected July 9th, 1959 late flowering stage.

Treatment Lbs P_2O_5/A	Well Drained Sites Field 1	Field 2	Field 3	Saline Sites Field 1	Field 2	Field 3
(i) Yield of dry matter (lbs/acre).						
0	3040	3344	2032	1824	1200	0.5
10	2944	2896	2160	2320	1552	7.5
20	2996	2480	2768	3120	2288	19.0
40	3248	3056	2736	3296	2288	21.0
60	2784	2656	2784	3776	3264	35.0
(ii) % P in plant (top growth only)						
0	.19	.17	.16	.15	.19	.11
10	.19	.14	.15	.16	.17	-
20	.19	.15	.15	.16	.16	.12
40	.19	.17	.16	.17	.14	.13
60	.20	.19	.18	.16	.14	.11

Table 2 - (cont'd)

(iii)	Yield of Phosphorus (lbs P/acre)					
0	5.78	5.68	3.25	2.74	2.28	.002
10	5.59	4.05	3.24	3.71	2.64	-
20	5.50	3.72	4.15	4.99	3.66	.02
40	6.17	5.20	4.38	5.60	3.20	.03
60	5.57	5.05	5.01	6.04	4.57	.04
B.	Final Yield of Grain (Cwt/Acre)					
0	17.29	15.89	9.48	11.53	15.13	0.07
10	17.82	18.43	8.79	12.33	16.98	0.03
20	17.40	19.57	9.95	17.39	19.76	0.56
40	18.48	20.23	8.19	19.42	21.91	0.70
60	18.77	21.25	9.26	24.11	25.53	0.72

Table 3. Analysis of variance, data from July 9th harvest date.

Source	Degree of Freedom	Yield Dry Matter Mean Square	Yield Phosphorus Mean Square
Replicates	1	21207.1	0.4489
Fields	2	15037703.9**	48.7320**
Fields x Replicates	2	222285.4	0.3702
Soil Type	1	18845131.9**	68.8867**
Soil Type x Fields	2	7414338.1	13.3671**
Soil Type x Replicate	1	129920.4	0.5171 *
Error a	2	143716.7	0.0249
Phosphate rate	4	943710 **	3.1730**
Phosphate rate x Fields	8	85904.5	0.7669 *
Phosphate rate x Soil type	4	833892.8**	1.1805**
Phosphate x Soil x Field	8	560937.3**	1.2835**
Error b	24	160972.8	0.2809
Total	59		

* - Significant at the 5% level of probability

** - Significant at the 1% (one %) level of probability

Table 4. Results of analyses of soil samples representing the plow layer from each site (1959).

Item	Orthic Black Sites Field 1	Field 2	Field 3	Saline Sites Field 1	Field 2	Field 3
$NaHCO_3$ extractable Phosphorus (ppm P.)	13.5	12.9	5.1	7.9	11.2	14.6
Conductivity of a Saturation extract	0.60	0.85	0.44	3.72	6.48	11.07
pH soil Paste	7.68	7.55	7.64	7.80	7.72	7.78
Inorganic carbon % C	0.24	1.45	1.13	0.33	3.12	0.62
% Yield (a)	93.6	78.5	115.7	59.4	69.1	10.0

(a) Yield of control expressed as a percentage of the yield from the 40 lb. P_2O_5 per acre treatment.

B. The effect of salinity on the growth and ash content of fertilized and non-fertilized barley.

It has frequently been noted that the germination and development of a cereal crop growing on saline soil is normal until the 3 to 4 leaf stage of growth. At this stage, salt damage, in the form of leaf tip burn and die back is frequently observed. It is also at this stage that plants receiving phosphate fertilizers are observed to be superior in growth and in freedom from salt damage to those in the control plots. Observations over a period of years suggest that the development of the difference in growth and symptoms of salt damage between fertilized and non-fertilized plants is dependent on the level of salinity, but the degree of this difference also varies from year to year, presumably depending on climatic factors. During the 1957 growing season, these phenomena were very evident on saline soils in Western Manitoba.

In the spring of 1957, a plot area was selected on a saline soil similar to that described in Materials and Methods.

Alternately fertilized and non-fertilized strips of barley (var. - Vantage) consisting of eight rows each, were seeded across the saline area. The individual rows were fifty- feet long and spaced nine-inches apart. The fertilizer treatment consisted of 11-48-0 applied at a rate calculated to give 20 pounds P_2O_5 per acre.

At three dates during the growing season, fourteen randomly selected samples were collected in pairs from adjacent fertilized and non-fertilized strips. The three sampling dates were May 27th (2 leaf stage), June 6th ($3\frac{1}{2}$ leaf stage), and July 10th (late bloom stage). Three feet of row, cut at ground level with shears, were harvested at each site and date. The yield of dry matter was determined by weighing the samples, after drying at 65° C in a drying oven. The ash content of the plants grown at each site was also determined, using the method described in Materials and Methods.

Soil samples were collected from each non-fertilized site on each date to determine the degree of soil salinity. The samples consisted of four, $1\frac{1}{2}$ inch cores, taken to a depth of four inches at randomly selected points in each sampling site. The cores from each site were mixed thoroughly and a sub-sample taken for conductivity measurements as described in Materials and Methods.

At the time of the first sampling there was no evidence of salt damage in the plot area. By the time of the second sampling date, leaf tip die back was severe on the control plots and moderate on the fertilized plots. The symptoms of salt damage had nearly disappeared by the third date. The average ash content of the fertilized plants (Table 5) was significantly below the control plants at the second sampling date and the yield of dry matter was much

greater from the fertilized sites. The ash content of the plants was not significantly affected by fertilizer at the earlier or at the later sampling dates.

These results suggest the possibility that salt damage is related to the accumulation of salts in the plant tissue and that the rate of accumulation is reduced if the plants are adequately supplied with phosphate. The damage being most severe at the three to four leaf stage of plant development. This stage of development corresponds to the development of the crown and secondary root system.

Table 5. Yield and ash content of barley grown on saline soil in 1957.

Item	Sampling Date May 27 fert.	May 27 control	June 6 fert.	June 6 control	July 10 fert.	July 10 control
Ash content (% fresh wgt.)	1.78	1.80	2.53	3.17	2.02	2.22
"t"	0.105		2.318*		0.591	
Degrees of freedom	6		6		6	
Dry weight grams/3' row	2.24	2.25	8.68	4.08	50.4	32.7
"t"	0.100		1.923		1.350	
Degrees of freedom	6		6		6	
Dry matter (mean of seven) % fresh weight	21.6	20.9	17.4	20.1	25.8	24.9
Mean conductivity saturation extract Millimhos / cm	9.39		7.04		6.76	
Range of conductivity measurements	4.75		5.58		2.73	

* Significant at the 5% level of probability.

C. The effect of a salinity gradient on the growth and phosphorus content of fertilized and non-fertilized cereal crops.

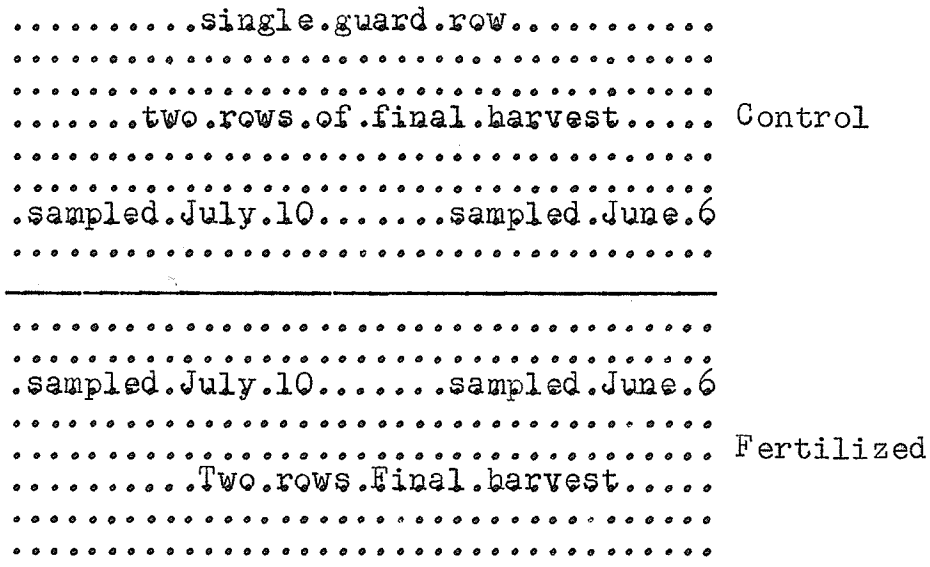
One of the features of saline soils which makes it difficult to obtain satisfactory experimental plots, is the high degree of variability in salt concentration found within very small areas. In 1958 and 1960 advantage was taken of this characteristic to determine if the phosphorus content and growth of fertilized and non-fertilized crops were directly dependent on the soluble salt concentration in the soil.

Plot areas were selected in the spring of 1958 and 1960 on saline soils, comparable to the description presented in Materials and Methods. In all instances these sites were located on summerfallow land.

In 1958, oats (Avena sativa, var. Rodney) were seeded in strips across the saline area. Each strip consisted of eight rows spaced nine inches apart, seeded with a v-belt seeder. Alternate strips were seeded with and without ammonium phosphate (11-48-0) fertilizer. The fertilizer was applied at a rate calculated to give twenty pounds per acre P_2O_5. Each strip was fifty feet long and a total of ten strips were seeded.

Plant samples were collected on June 6th (three leaf stage), July 10th (early blossom stage) and August 5th (crop mature), for yield and phosphorus determinations. Five paired sampling sites were selected to give as wide a salin-

ity gradient as possible. Plant samples were clipped at ground level on each occasion, according to the following scheme:



Soil samples were collected on the June 6th sampling date using a 1½ inch core sampler. A total of ten, 0 - 4" borings were collected from the control plot at each of the five sites and bulked for analytical purposes.

A similar procedure was followed in 1960 except that wheat (var. Selkirk) was used as a test crop and the treatment consisted of:

a. Control (10 lbs. N/acre).

b. Fertilized (10 lbs. N plus 40 lbs. P_2O_5/acre).

It was found that the salinity levels were not as high in 1960, due presumably to leaching by heavy rains in the fall of 1959 and spring of 1960. Plant and soil samples

were collected on June 16th, which corresponded to the three leaf stage of growth, using similar methods to those described for oats in the 1958 experiment.

At the June 6th sampling date in 1958 the yield of dry matter, the yield of plant phosphorus and the percentage plant phosphorus (Table 6) all decreased with increasing soil salinity in the fertilizer treated plants. These factors were not related to soil salinity in the control plants at this harvest date. This suggests that the ability of the plant to absorb fertilizer phosphate decreases with increasing salinity. On the other hand, the results from the July 10th sampling indicate that the yield of dry matter and phosphorus decreased more rapidly with increasing salinity with the controls than with the fertilizer treatment. This relationship is more readily explained by the assumption that the ability of the plant to absorb soil phosphate decreases with increasing salinity while the availability of the fertilizer phosphate is not affected by the presence of salts. This contradiction is difficult to explain by a single mechanism and probably results from the interaction of a number of factors.

The percentage increase in the final yield of grain (Table 6 D.) resulting from fertilizer is much greater on the saline than on the non-saline soil as in the experiments reported in Section I.

Table 6. The effect of a salinity gradient on phosphorus uptake and yield of oats (1958).

Sample Date	Treatment	Conductivity Millimhos/cm 9.54	8.45	7.93	6.52	3.96	Non-Saline Site in Same Field
A. Yield of dry matter. (g/6 foot of row)							
June 6th	fert.	5.85	6.66	6.46	7.91	15.11	
	control	2.85	3.29	4.36	4.88	3.80	
July 10th	fert.	66.6	123.9	71.4	111.6	216.0	
	control	15.9	28.3	21.6	63.0	104.2	
B. Yield of phosphorus. (mg P/6 foot row)							
June 6th	fert.	17.2	21.2	21.3	23.5	56.7	
	control	5.6	4.1	6.7	6.3	7.4	
July 10th	fert.	119.9	219.1	130.7	181.9	276.2	
	control	31.1	31.8	42.1	91.2	153.0	
C. Percentage phosphorus in plants							
June 6th	fert.	0.29	0.32	0.33	0.30	0.38	
	control	0.20	0.13	0.15	0.13	0.20	
July 10th	fert.	0.18	0.18	0.18	0.16	0.13	
	control	0.20	0.11	0.20	0.15	0.15	
D. Final yield of grain (bu./acre)							
	fert.	28.6	23.0	27.5	38.8	59.0	83.0
	control	2.7	8.9	8.2	22.9	20.5	61.5
Increase in final yield as % of control		959.2	158.4	235.6	69.4	187.5	39.8

From the data given in Table 7 it is clear that the yield of dry matter of wheat grown on non-fertilized soil is negatively correlated with salinity level while the yield of wheat grown on fertilized soil is not correlated with salinity. The relation between the increase in yield due to phosphate and the degree of soil salinity approaches the level of positive correlation required for significance. The percentage phosphorus in the plants seeded with fertilizer tends to increase with increasing salinity, while the level of salinity had no consistent effect on the phosphorus percentage in the non-fertilized plants at this stage of growth.

The amount of soil phosphate extractable with ammonium fluoride-hydrochloric acid is not correlated with the salinity level. Again the data suggest that increasing salinity decreases the availability of soil phosphate without affecting the availability of fertilizer phosphate. However, as in the previous experiments the extractant used to determine available soil phosphate failed to confirm this.

Table 7. The effect of a salinity gradient on phosphorus uptake and yield of wheat (1960).

Conductivity Millimhos per cm	Dry weight milligrams per plant 10-40-0*	10-0-0**	Increase due to Phosphate	% P 10-40-0	10-0-0	Milligrams P/plant 10-40-0	10-0-0	NH_4F-HCl extractable Phosphate PPM.P
0.81	.167	.132	.035	.266	.250	.444	.330	7
0.89	.127	.102	.025	.274	.185	.348	.189	13
1.06	.142	.074	.068	.270	.160	.383	.118	34
1.25	.134	.059	.075	.300	.185	.402	.109	16
1.55	.157	.057	.100	.290	.220	.455	.125	11
1.56	.146	.092	.054	.320	.195	.467	.179	4
2.22	.161	.059	.102	.290	.150	.467	.089	10
2.56	.111	.040	.071	.300	.160	.333	.064	10
correlation with conductivity	-.292	-.758	.617	.564	-.061	-.027	-.702	-.293

r = 0.666 significant at 5% level of probability

* 10-40-0 - Fertilizer treatment 10 lbs of nitrogen and 40 lbs of P_2O_5 per acre respectively.

** 10-0-0 - 10 lbs per acre of nitrogen without phosphate.

II. Greenhouse Studies. Comparisons of the effect of individual salts on phosphorus uptake and plant growth in artificially salinized media.

The field experiments indicated that the effect of phosphate fertilizer on cereals was evident at a much earlier stage of growth, in saline than in non-saline soil. This suggested that it would be possible by using short term greenhouse studies, to determine if the presence of soluble salts in the soil affected the quantity of fertilizer phosphate absorbed by the plants as was indicated by the experiment reported in Section I C. To accomplish this a growth medium containing a very limited amount of available phosphate and with a high capacity to adsorb and precipitate added soluble phosphate was desirable. The mixture of calcareous sand and vermiculite described in Materials and Methods was used because of these properties.

To make the greenhouse experiments as comparable as possible with the field studies, granular fertilizer was used. The fertilizer was prepared by selecting the granules of ammonium phosphate fertilizer (11-48-0) which collected in the mesh of a 2 mm sieve, to obtain as uniformly sized granules as possible. This was necessary to make the dissolution rate and the rate of application as uniform as possible.

Preliminary experiments showed that it was extremely difficult to work with saline soils under local greenhouse conditions because due to evaporation the salts accumulated

at the soil surface between waterings, also because of the poor soil structure and aeration resulting from salt treatments. These difficulties were overcome to a large extent by using the mixture of sand and vermiculite as a growth medium in 1 gallon plastic pots covered with perforated polyethylene film.

The treatments were arranged on a greenhouse bench in a split-plot design using two replicates. The experiment was repeated three times during the winter (1959 - 1960) to make a total of six replications of the treatments. The first two replicates were grown between November 24th and December 15th, 1959, the second pair from January 17th to February 3rd and the last two between March 1st and 23rd, 1960. All replicates were grown in a greenhouse compartment with the temperature controlled at 15° C but light conditions varied considerably during the different periods.

The major treatments consisted of four salts applied to the potting mixture at the three rates of application shown in Table 8 A.

It was anticipated that it would be difficult to apply the four salts at rates of application which would be comparable from the standpoint of chemical activity, consequently the growth medium from each pot was saved and the specific conductivity of a saturation extract of this material determined. The results of these determinations are given in Table 8 B expressed in units of millimhos per centimeter.

Table 8. The rates of application of salts and the specific conductivity of a saturation extract of the potting mixture after harvest.

Treatment Number	$MgCl_2.6H_2O$	NaCl	$MgSO_4.7H_2O$	$Na_2SO_4.10H_2O$
A.	Rate of salt application (% of dry weight of potting mixture).			
1	0.35	0.10	0.42	0.55
2	0.70	0.20	0.84	1.10
3	1.05	0.30	1.26	1.65
B.	Specific conductivity (millimhos / cm) of a saturation extract of potting mixture.			
1	12.27	6.24	5.62	8.12
2	19.33	11.11	9.41	15.06
3	29.40	15.87	12.48	21.01
Control	0.85			

Two subtreatments were used:

1. Control (no fertilizer).

2. Four granules of 11-48-0 fertilizer per seed (approximately 10 lbs per acre P_2O_5).

Sufficient potassium nitrate was added to each pot to supply the equivalent of 80 lbs per acre N, on an area basis.

The pots were prepared as described in Materials and Methods and 10 uniformly swollen barley seeds were placed in ½" holes pressed into the potting mixture in each pot. Four granules of fertilizer were placed with each seed in the treated pots and each pot was covered with a clear 2 ml polyethylene film, cut to fit the soil surface. As the shoots emerged, holes were punched in the plastic to allow shoot development. Representative pots were weighed at regular intervals and water added as needed to maintain the original weight. The plants were allowed to grow until the third full leaf stage when all plants were harvested as described in Materials and Methods.

The analysis of variance (Table 10) indicates a highly significant difference in yield of dry matter between plants grown within treatments or between replicate pairs. This difference is probably due to difference in light, and other uncontrolled environmental factors. There is also a highly significant difference in yield resulting from the different salt treatments. The conductivity of saturation extracts of the media receiving the various treatments (Table 8 B), indi-

cates that the rates of application of the different salts are not comparable. These differences are large and may have resulted from a differential effect of the calcareous substrate on the various ions or from losses occuring during watering. If it is assumed that the conductivity of a saturation extract is the best criterion of the effective concentration, then the effect of different salts can only be compared by the use of covariance procedures. When analysed by this procedure (19), the "F" value for comparisons of the means of the four salts falls below the level required for significance. This could not be interpreted as evidence of equivalent effects of the various salts, but it does indicate that this experiment fails to prove that differences do exist between the four salts tested.

The rate of salt application and the use of phosphate fertilizers both had a highly significant effect on dry weight production by the barley plants. In general, increasing the salt concentration decreased dry weight production, although the lowest rate of application of sodium chloride is seen to have increased dry weight production above the level of the control. The application of phosphate fertilizer consistently increased dry weight production by the plants. The magnitude of the increase was significantly influenced by the concentration of salt associated with it.

Table 9. The effect of three levels of four salts on growth and phosphorus absorption.

A. Milligrams dry weight per plant (mean 6 replicates).

Treatment	$MgCl_2$ fert.	$MgCl_2$ control	NaCl fert.	NaCl control	$MgSO_4$ fert.	$MgSO_4$ control	Na_2SO_4 fert.	Na_2SO_4 control
Rate of Salt								
1	123.9	86.8	162.0	108.0	126.3	92.9	115.7	81.0
2	75.5	58.0	114.3	89.1	81.6	75.5	74.3	63.2
3	37.9	33.0	81.1	70.3	75.9	53.8	49.5	41.4
No added salt	146.1	105.8						

B. Percentage P in plants. (mean 6 replicates)

1	.456	.148	.548	.147	.555	.157	.508	.165
2	.388	.168	.416	.137	.527	.167	.477	.186
3	.339	.229	.315	.153	.456	.205	.422	.219
No added salt	.427	.125						

C. Milligrams P per plant (mean 6 replicates).

1	.565	.128	.888	.159	.701	.146	.588	.134
2	.303	.097	.477	.122	.430	.126	.354	.110
3	.128	.076	.255	.107	.346	.110	.209	.091
No added salt	.624	.132						

D. original seed

Milligrams P. per seed = 0.127

Milligrams dry weight per seed = 30.7

Table 9 (continued)

E. Percentage dry matter

Rate of salt								
1	12.1	12.1	11.7	11.2	10.4	11.8	10.4	11.3
2	16.2	16.9	11.7	11.5	13.4	12.8	12.1	11.6
3	21.1	22.2	12.0	11.7	18.2	18.1	13.5	13.4
No added salt	12.5	12.3						

Table 10. Analysis of variance of data from Table 9 A (milligrams dry weight per plant).

Source	Degrees of Freedom	Mean Square
Runs	2	6544.19
Replicates	1	1049.22
Runs x Replicates	2	42.13
Chemicals	3	9555.00**
Chemicals x Runs	6	827.93
Chemicals x Replicates	3	425.51
Chem. x Runs x Reps.	6	321.19
Rates of chemicals	2	38361.325**
Fertilizer	1	17084.67 **
Rates x Runs	4	3023.52 **
Rates x chemicals	6	382.537*
Rates x Fertilizer	2	2689.01 **
Fertilizer x Runs	2	1413.455**
Fertilizer x Chemical	3	259.875
Remainder	100	181.824
Total	143	

* significant at 5% level of probability.

** significant at 1% level of probability.

In general, the magnitude of the fertilizer effect on yield decreased with increasing salt concentration. The relationship is not linear however, and the effect of fertilizer phosphate on growth increased between the control and the lowest rate of salt application and then decreased at higher salt concentrations.

In part B of Table 9 it is clear from the data that increasing salt concentration had an opposite effect on the percentage phosphate in the plant depending on whether fertilizer was applied or not. The percentage phosphate in the plant decreased with increasing concentration when phosphate fertilizer was applied and increased with increasing concentration in the control pots. Since phosphate uptake is dependent on the growth rate this relationship in itself is not evidence that the presence of increasing amounts of salt affects the absorption of phosphate independently of the growth rate. Consequently the amount of phosphate absorbed per plant during the growth period per unit gain in dry weight is presented in Table 11. From these data it is seen that in all cases, an increase in the rate of salt application increased the relative absorption of phosphate at the lower rates and decreased it at higher rates. From Part C of Table 9 and Part B of Table 11 it is seen that at the higher levels of salt application non-fertilized plants were unable to maintain the original level of phosphate carried by the seed. These data suggest that under saline conditions barley

plants and perhaps cereal crops, in general, can lose appreciable quantities of phosphate to the growth medium. However, since complete recovery of plant roots from the sand-vermiculite growth medium was not certain, further evidence of this phenomenon was obtained using liquid media and shorter growth periods. These experiments will be described in Section III of this thesis.

Table 11. Milligrams phosphorus uptake by barley plants per milligram gain in dry weight x 100.

A. Fertilized

Rate	$MgCl_2$	NaCl	$MgSO_4$	Na_2SO_4
1	.470	.580	.600	.542
2	.390	.419	.595	.521
3	.014	.254	.485	.436
Control	.367			

B. Non-fertilized

Rate	$MgCl_2$	NaCl	$MgSO_4$	Na_2SO_4
1	.0018	.032	.019	.014
2	-.136	-.009	-.002	-.052
3	-2.21	-.051	-.074	-.337
Control	.0066			

The gross effect of increasing salt concentration on the ability of barley plants to accumulate fertilizer phosphate can be represented by $\triangle$ P (the difference between the number of milligrams of phosphorus per plant grown with fertilizer phosphate and without it, at the same salt level). Comparing Part C of Table 9 and Table 8 B it is seen that $\triangle$ P tends to increase with increasing salt concentration at low salt levels and decrease at high levels. Similar results were obtained in the field experiments reported in Table 6 and 7. Since harvesting in all cases was at approximately the same stage of growth, the data are comparable. Figure 2 shows all of the individual measurements from these three experiments with $\triangle$ P (milligrams P per fertilized plant - milligrams P per non-fertilized plant) as the ordinate and the conductivity of a saturation extract of the soil, as the abscissa.

The data appear to have a definite maximum at a value of about 4 millimhos conductivity. Observation of the data suggest that it is best described by two linear functions. The two lines appearing in Figure 1 were located by visual estimate, and have the approximate equations:

$$y = 0.350 + 0.050\ x) \begin{matrix} 3.56 \\ 0 \end{matrix}$$

and

$$y = 0.625 - 0.272\ x) \begin{matrix} 23.00 \\ 3.56 \end{matrix}$$

Where y = $\triangle$ P and x equals the specific conductivity.

The two lines intersect at x = 3.56 providing the limiting terms in the equations.

Using these two functions to estimate $\triangle$ P the standard error of estimate (29) is 0.999, which is 38.2% of the mean. This implies extreme variation from regression which is not surprising since the data were derived from four different experiments and are directly dependent on the stage of growth at which samples were collected.

The results of these experiments indicate that the presence of soluble salts in the growth medium influence the plants ability to accumulate phosphate independently of their effect on growth. However, since growth and phosphate accumulation are correlated and mutually dependent factors the resultant functions are complex and difficult to interpret.

The results also indicate that the ionic strength of the solution is relatively more important than the identity of the particular salt. However, the data in Table 11 Part A suggest that the chloride ion has a much greater effect on phosphate accumulation than the sulphate ion. Further experiments at a greater number of rates of application would be necessary to confirm this distinction.

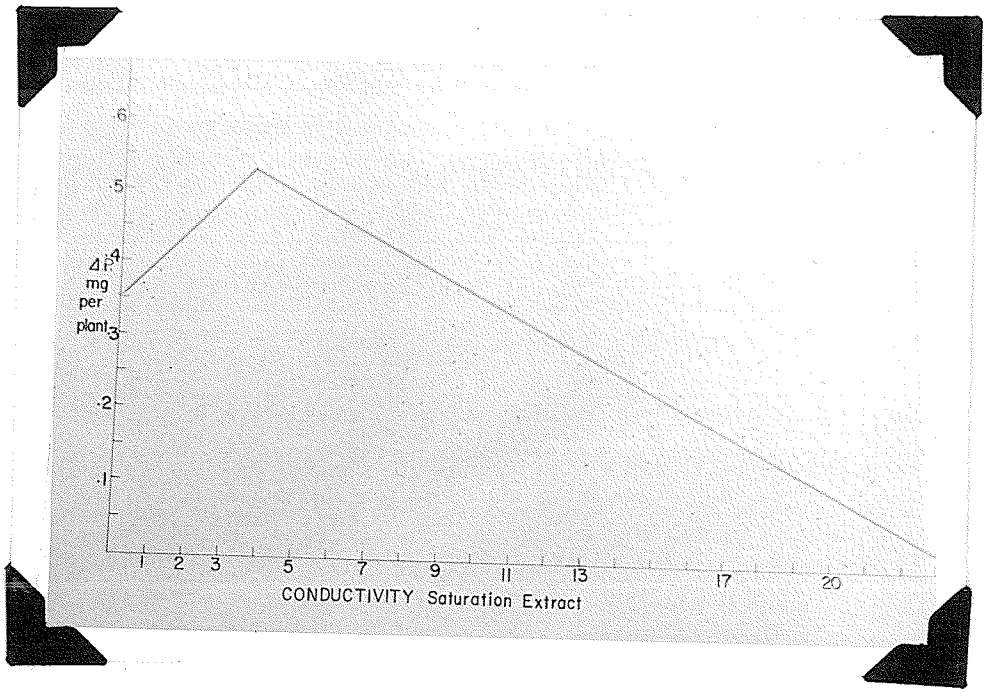


Figure 2. The relation between △ P (mg P per fert. plant - mg P per cont. plant) and the conductivity of a saturation soil extract (millimhos per cm.).

III Phosphorus absorption and retention by barley in liquid culture.

A. The effect of a saline solution on phosphorus absorption by barley root segments.

The field experiments and the experiment conducted in the greenhouse (Section I and II), indicated that phosphorus absorption by cereal crops was reduced by excessively saline soil. The most widely accepted explanation of the nutrient absorption mechanism (28, 38, 46), postulates the existence of carrier molecules which transport ions across a diffusion barrier within the plant cell. Kinetic studies (27) have indicated that similar ions are frequently mutually competitive in the transport process and the absorption of one is reduced in the presence of another. To determine if this mechanism was responsible for the observed reduction in phosphate absorption in saline soil, an experiment was conducted using P^{32} to measure the rate of phosphate absorption by barley root segments in saline and non-saline solutions.

Barley from the seed source, described in Materials and Methods was washed in distilled water and placed in germinating trays for twenty-four hours. Six uniformly swollen seeds were then inserted in holes which had been drilled in freshly waxed cork discs, slightly smaller in diameter than the culture tubes. These discs were floated on the solution in the culture tubes, the level of the liquid being held so the corks floated at the shelf level in the cabinet. This

prevented light from entering the lower portion of the cabinet. Each tube contained approximately 150 ml of culture solution.

Barley plants were grown for two weeks in the culture tubes (as described in Materials and Methods) in a growth chamber constructed in the Botany Department of the University of Manitoba. The temperature of the growth chamber was maintained at 16° C during the growth period and the 3 hour absorption period. The solutions in all of the culture tubes were changed daily. One half of the culture tubes contained 1 x Hoagland's solution throughout the experiment and the concentration of the solution in the remainder of the culture tubes was increased daily commencing with 1 x Hoagland's solution and using saline solutions 1 to 7 successively. Number 7 saline solution was used from the eighth day of growth until the end of the experiment. The absorption procedure and the radioactive assays were conducted as described in Materials and Methods.

During the 3 hour absorption period the treatments were as follows:

1. 1 x Hoagland's without phosphate.
2. " 10^{-6}molar with respect to KH_2PO_4+P^{32}.
3. " 10^{-6} " " " " " " .
4. " 10^{-4} " " " " " " .
5. #7 Saline without phosphate.

6. #7 Saline 10^{-6} molar with respect to KH_2PO_4+P^{32}.
7. " " 10^{-5} " " " " " " .
8. " " 10^{-4} " " " " " " .

This experiment was repeated three times and the mean results of the phosphate absorption measurements appear in Table 12.

Table 12. Phosphorus uptake by two week old barley roots during a three hour absorption period.

Solution	Substrate concentration molar P			
	10^{-6}	10^{-5}	10^{-4}	Mean
	ugrams P per 20 plants			
1 x Hoaglands	0.24	2.81	7.93	3.66
#7 Saline	0.16	1.93	8.63	3.58

Standard error of a mean = 0.462

The amount of phosphorus absorbed in this experiment was not significantly affected by the composition of the basal culture solution, but the concentration of phosphate in the substrate did significantly affect the rate of phosphorus accumulation. The very large range in the order of magnitude of the results makes statistical interpretation difficult because the standard error is large relative to the values obtained at the low rates of application. Using 10^{-6}M phosphate as substrate the values for replicates ranged from 0.15 to 0.29 and from 0.13 to 0.29 respectively for 1 x Hoagland's and #7 saline. Consequently no significance can be placed on the large difference between the mean values obtained for these two solutions.

B. The effect of a saline solution on phosphorus absorption by barley plants.

The results of phosphorus absorption studies using barley root segments (Section A) appeared to contradict the field and greenhouse studies reported in Section I and II. Further experiments were conducted using complete plants and longer absorption periods to determine if this contradiction was caused by the use of liquid media or by the technique of measurement.

This experiment was conducted in the Growth Cabinet described in Materials and Methods. The procedure was identical to that described in Section III A. until 72 hours before the harvest time. During the final 72 hours of

growth the treatments were as described in Section III A, except that P^{32} was not added. These solutions were changed every 24 hours until the plants were harvested.

At the end of the 72 hours absorption period, after 14 days of growth, the plants were removed from the cork discs, washed with running distilled water and blotted dry with filter paper. The fresh weight, dry weight, and total phosphorus per plant were determined for each treatment by the procedures described in Materials and Methods. The experiment was repeated three times.

Table 13. The effect of solution composition and phosphate concentration on phosphorus uptake by barley plants (72 hours absorption period with whole plants).

Solution	Concentration P molarity				
	0	10^{-6}	10^{-5}	10^{-4}	Mean
	milligrams P per plant				
1 x Hoagland's	.1239	.1347	.1418	.1549	.1388
#7 Saline	.1141	.1211	.1270	.1310	.1234

Standard error of a mean (solutions) = 0.0008

" " " " " (rates) = 0.0011

Phosphorus accumulation by barley plants (Table 13) was significantly lower when grown in saline solutions than in normal nutrient solution. This does not agree with the results obtained using radioactive isotope techniques (Table 12) to measure phosphorus absorption. This suggests that the process of permanent accumulation of phosphorus by barley plants may be distinct from the cellular absorption mechanism. The results in Table 12 are not conclusive however, because of the possibility of isotopic exchange between the very dilute phosphate solutions and the endogenous plant phosphate. This would be a very serious error, particularly if the amount of exchange was affected by treatment.

C. The effect of solution concentration and temperature on the phosphorus content of two week old barley plants.

The results of the greenhouse experiments (Section II) indicated that significant losses of plant phosphorus might occur under unfavorable environmental condition, and particularly in saline soils. However, the difficulties inherent in harvesting plant roots from solid media limited the confidence placed in these findings.

To confirm these results an experiment was conducted using liquid media in the growth chamber described in Materials and Methods. Barley plants were grown for two

weeks using methods identical with those described in Section III A. At the end of the two-week growth period the plants were harvested and analysed for fresh weight, dry weight, and total phosphorus content.

This procedure was repeated twice with the cabinet temperature controlled at each of four temperatures (12, 14, 16 and 18°). Four solutions were used during each growth cycle, #1 and #7 saline solutions and 1x and 8x Hoagland's solution. Each solution occupied twelve culture tubes in the cabinet and the plants removed from each tube were bulked with the plants from five other tubes from the same solution. Consequently the results presented in Table 14 are the mean of four analyses, two of which were run at the same time. The results are expressed in terms of mg P. per plant and the phosphate content of the original seed is included for comparative purposes.

The phosphate content of the barley was significantly less at 12° C than it was at 18° C. Since no phosphate was added to the solutions this implies a direct loss of plant phosphate to the solution. The mean loss from plants grown at 12° C amounted to 20% of the original seed phosphate. The mean differences between solutions were not significant, but the interaction between solutions and temperatures was significant at the 5% level of probability.

In general the data presented in Tables 2, 11 and 14 in-

dicate that at low temperatures significant losses of plant phosphorus occur and that this loss is greater under saline conditions.

Table 14. The effect of solution composition and temperature on the phosphorus content of barley.

Solution	Temperature (degrees centigrade) 12	14	16	18	Mean
	milligrams P per plant				
1 x Hoagland's	.1005	.1191	.1186	.1238	.1155
⅛ x Hoagland's	.1085	.1025	.1119	.1233	.1115
#1 Saline	.1032	.0983	.1173	.1259	.1112
#7 Saline	.0942	.1030	.1128	.1325	.1106
Mean	.1016	.1058	.1152	.1264	

Original seed (mg P/plant) = 0.1271

Standard error of a mean (temperature) = .005

Standard error of a mean (solutions x temp.) = .006

IV Metabolic Studies.

A. The respiratory rate of barley root segments grown in saline solutions.

Reports of phosphorus loss by plant roots have appeared in the literature (30, 64, 71, 79), and have generally been attributed to reduced respiratory activity. To determine if the phosphorus losses which occured in saline solutions (Table 14) could be attributed to this cause, the oxygen uptake by barley root segments grown in #7 saline solution and Hoagland's nutrient solution were determined.

Barley plants were grown in the culture tubes as described in Section III A. On the fourteenth day, the plants were harvested and the rate of oxygen uptake by root segments measured as described in Materials and Methods.

The cabinet temperature was held at 16° C during the growth period and the oxygen uptake determinations were conducted at the same temperature. Three independent measurements from each solution were made on root segments grown during two separate periods, giving six comparisons of oxygen uptake by roots grown in #7 saline solution with those grown in normal Hoagland's solution.

The respiratory rate per plant (Table 15) was significantly less in the saline solution than in Hoagland's solution, but the respiratory rate tends to be higher in the saline solutions when they are compared on the basis of dry weight. Salinity appears to have a relatively greater effect on dry weight production than on respiration rate.

Table 15. The effect of a saline solution on the oxygen uptake of two week old barley roots.

Solution	O_2 Uptake ul/hr. per g dry wgt.	O_2 Uptake ul/hr. per plant	% dry matter
1 x Hoagland's	559.6	0.92*	14.0
#7 Saline	587.8	0.65*	14.4

* Difference significant at 5% level of probability.

B. The cytochrome oxidase activity of the particulate fraction from barley roots grown in saline solutions.

Cytochrome C is an important intermediary in the principal terminal oxidase system in the plant cell. The cytochrome system is also considered to be the means of nutrient absorption in an important contemporary hypothesis (65). It has also been reported that increasing concentration of monovalent cations increase the activity of particulate cytochrome oxidase from barley roots (72). This was observed to attain a maximum and decrease at higher concentrations. It appeared, therefore, that determinations of the comparative activity of cytochrome oxidase from plant roots grown in a saline solution would be a valuable supplement to the studies of growth, phosphate absorption and respiration.

The barley plants were grown for two weeks following the procedures outlined in Section III A. Cytochrome oxidase activity was measured by the method described in Materials and Methods.

The determinations were conducted in duplicate on suspensions prepared from plants grown in 1 x Hoagland's, 8 x Hoagland's, #1 Saline and #7 Saline solutions. The growth procedure was repeated three times making a total of six readings per solution. The change in optical density per gram of root tissue was estimated from the shape of the best fitting linear function with the variables time and optical density (Table 16A).

The results of the experiment indicate that the cytochrome oxidase activity of the particulate fraction from barley roots is not significantly influenced by a saline growth medium, when compared on the basis of the fresh weight (Table 16B). However, when they are compared on the basis of the total nitrogen content of the particulate fraction, the roots grown in a saline medium show significantly less activity than those grown in normal nutrient solution (Table 16C). Since the fresh weight of root per plant was reduced by the saline solution, the cytochrome oxidase activity per plant would also be less when grown in saline as opposed to Hoagland's nutrient solution. If the material isolated for the cytochrome oxidase studies was exclusively mitochondrial then it follows that the concentration of mitochondria was higher in the plants grown in saline media. It is also possible however that cytoplasmic protein was not completely removed during the fractionation of the roots grown in saline media.

The measurements of respiration (Table 15) and cytochrome oxidase activity (Table 16) indicate that the general level of metabolism in the barley roots is not specifically reduced by saline conditions but since growth is reduced the effective activity per plant is reduced.

Table 16 A. The effect of a particulate suspension from barley roots on the optical density of cytochrome C. (mean 3 determinations).

Time Minutes	1 x Hoagland's	⅛ x Hoagland's	#1 Saline	#7 Saline
0	.635	.630	.628	.627
1	.584	.581	.567	.576
2	.574	.558	.542	.566
3	.560	.548	.517	
4	.553	.545	.503	.553
5	.537	.527	.487	.548
10	.481	.490	.414	.509
15	.442	.445	.368	.446
Completely Oxidized (Ferricyanide)	.222	.208	.226	.225

Table 16 B. Change in optical density per gram fresh plant material per minute.

Culture Media	1 x Hoagland's	⅛ x Hoagland's	#1 Saline	#7 Saline
Change in optical density	1.72	1.96	2.18	1.99

Std. dev. = 0.36

Table 16 C. Change in optical density per milligram nitrogen per minute.

Culture media	1 x Hoagland's	8 x Hoagland's	#1 Saline	#7 Saline
Change in optical density	2.35	2.12	1.99	0.92

Standard error of a mean = 0.59

V. The apparent free space of barley roots as affected by solution concentration.

The most widely accepted concept of the mechanism responsible for nutrient absorption distinguishes between a passive (adsorbed) component and an active (absorbed) component (8, 15, 28, 65). The adsorbed component results from purely physical factors such as ionic exchange and simple diffusion. The volume of the plant tissue occupied by this adsorbed phase has become known as the free space (8). The term apparent free space was adopted because the free space cannot be measured directly and is estimated in terms of the amount of some readily diffusible substance which can be allowed to equilibriate with the free space solution. According to this concept the total volume of a plant cell is composed of the free space and the osmotic volume.

The osmotic inhibition theory (41) attributes the decrease in plant growth which occurs in saline soils to the effect of the osmotic pressure of the substrate on the rate of expansion of the osmotic volume of the plant tissue. Since this volume concept is basic to the theory of the effect of salinity on plant growth and is also an important distinction in nutrient absorption studies, an experiment was conducted to determine if the apparent free space of barley roots was affected by the concentration of the nutrient solution.

The determinations were made in duplicate on plants grown in 1 x Hoagland's, 4 x Hoagland's, and 8 x Hoagland's solution following the procedures outlined in Section III A and in Materials and Methods. The experiment was repeated three times.

The apparent free space of barley roots increased with increasing substrate concentration (Table 17). If the osmotic volume is the principal location for the accumulation of nutrient ions (39, 64), a reduction in this volume would be expected to cause a reduction in nutrient absorption.

Table 17. Apparent free space of barley roots grown in nutrient solution of increasing concentration.

Solution	Apparent % Free Space
1 x Hoagland's	7.4
4 x "	14.3
8 x "	19.9

Standard error of a mean = 2.2

VI Fractionation of phosphorus compounds in barley plants.

Phytin (the Ca-Mg salt of inositol hexaphosphate) is the principal phosphate ester found in the seed of cereals. The rate of hydrolysis of this compound relative to the rate of metabolic phosphorylation in the growing plant would control the concentration of inorganic phosphate, during the early stages of growth. A high concentration of diffusible (presumed to be inorganic) phosphate in the plant would increase the phosphate diffusion gradient between plant and substrate. This might be partially responsible for the phosphate losses reported in Section III C.

The phosphorus content of the following fractions from (I) barley seed, (II) seed that had been soaked for 24 hours, (III) two week old barley plants, was determined.

Fraction

1. Total phosphorus.
2. Lipid " .
3. Total acid soluble phosphorus.
4. Inorganic phosphorus.
5. Phytin " .
6. Ester " .
7. Protein " .

In all cases the barley was from the source described in Materials and Methods. The method of seed preparation (24

hours) and the fractionation procedure were also described in Materials and Methods.

The two week old barley plants were grown at two temperatures (12 and 18° C) in a constant temperature water bath as follows:

The temperature was controlled in the water bath by continuously circulating water through a copper coil in a refrigerated bath and setting the thermostat in the water bath to control the temperature at the desired level. The returning water from the cooler was injected below a perforated stainless steel support which held the beakers. Manual temperature checks indicated that the temperature variation within the bath never exceeded 0.5° C.

During operation the bath contained twelve beakers containing 400 ml of sterile vermiculite saturated with either 1 x Hoagland's solution or #7 Saline solution. Each beaker was weighed at the beginning of the growth period and the weight maintained with distilled water.

Thirty barley seeds from the source described previously were placed in each beaker with tweezers. The water bath was then covered with a clear polyethylene cover which maintained a high level of humidity in the bath and reduced water requirements.

Light was supplied by four 15-watt fluorescent tubes supported directly above the plastic cover. These lights were operated continuously and in addition, during daylight hours,

indirect light entered the chamber through the plastic cover.

At the end of the two-week growth period the beakers were removed and the root mass plus the vermiculite was shaken out of the beaker. The excess vermiculite was carefully removed from the roots and the roots were washed free of vermiculite in running water. The roots and tops from three beakers of a single solution were counted and combined for analysis. After counting the plants were blotted dry with paper toweling, weighed and cut into short segments with shears. They were then frozen in lyophilizing bottles and dried while frozen, under vacuum. When dry they were again weighed and ground in a porcelain mortar.

The phosphorus content of the various fractions was then determined as described in Materials and Methods. The results of this experiment appear in Table 18 (seeds) and in Table 19 (2 week old plants).

The phosphorus content of the inorganic fraction of the seed was 10.5% of the total phosphorus, this fraction was 22.6% of the total following 24 hours soaking, and at two weeks the inorganic fraction accounted for approximately 70% of the total plant phosphorus. This gain in inorganic phosphate was due principally to a reduction in the phytin fraction.

The complete lack of an ester fraction in the two week old barley plants (Table 19) as compared to the seed is probably due to inadequacies in the fractionation procedure.

Table 18. Mean phosphorus content of the various fractions in six, 200-seed replicates of barley.

	Original Seed		24 hour Soaking	
	% P	St. Dev.	% P	St. Dev.
Total phosphate	0.408	0.013	0.406	0.012
Lipid phosphate	0.018	0.003	0.013	0.002
Total acid soluble phosphate	0.305	0.013	0.273	0.036
Inorganic phosphate	0.043	0.005	0.092	0.009
Phytin phosphate	0.167	0.006	0.058	0.008
Ester phosphate	0.095		0.124	
Protein phosphate	0.084		0.120	
Dry weight (g per plant)	0.0310	0.0013	0.0333	0.0021

Table 19. Percentage phosphate contained in the various phosphate fractions in two-week old barley seedlings.

Phosphate fraction	Grown at 12° C in 1 x Hoag. % P	Grown at 12° C in #7 Saline % P	Grown at 18° C in 1 x Hoag. % P	Grown at 18° C in #7 Saline % P
Total	.399	.478	.305	.371
Lipid	.037	.040	.025	.025
Total acid soluble	.289	.364	.232	.293
Inorganic	.280	.353	.210	.252
Phytin	.019	.022	.027	.033
Ester	-.009	-.011	-.005	.008
Protein	.072	.074	.073	.047
Residue after total acid extract	.084	.096		
Grams dry weight per plant	.0297	.0259	.0392	.0303
Inorganic phosphate mg P/plant	.083	.092	.082	.077

Hydrolysis during extraction is probably higher in the older plant tissue and the phosphate from the labile esters would be extracted with the inorganic fraction. This is probably not a serious limitation since the purpose was to illustrate the effect of the treatments on the concentration of the readily diffusible phosphate fraction.

The percentage phosphorus in the inorganic fraction was increased by low temperatures and by salinity. This increase was due to the effect of these factors on growth, presumably the degree of hydrolysis was not influenced by treatment while the amount of growth was reduced, consequently the percentage phosphorus in the inorganic fraction increased.

A number of authors have reported that the rate of nutrient absorption by plants is dependent on the concentration of the nutrient within the plant tissue. Since the data presented in Table 19 indicate that the concentration of phosphorus is higher in plants grown under saline conditions, it is possible that the increased concentration gradient could cause reduced phosphate absorption under saline conditions and at low temperatures. The tendency for diffusion of phosphate from the plant root to the medium would also be dependent on the concentration gradient, which is increased by salinity and low temperature.

GENERAL DISCUSSIONS AND CONCLUSIONS

The results of the experiments reported in Tables 1, 2, 5, 6, and 9, indicate that the plant response obtained from phosphate fertilizers and the amount of phosphate absorbed by fertilized plants is related to the level of soil salinity. This observed relationship could be the result of a number of factors.

1. The presence of soluble salts in the soil might decrease the solubility of soil phosphate and thus decrease it's availability to plants.

2. The presence of soluble salts might also alter the rate of dissolution, adsorption or precipitation of fertilizer phosphate and thus alter the amount absorbed by the plant.

3. The soluble salts might conceivably disturb the normal operation of the plants phosphate absorption mechanism.

4. The possibility also exists that the presence of soluble salts could have an indirect effect on the ability of the plant to absorb and retain phosphate.

Chemical theory on the effect of neutral salts on the solubility of less soluble materials (96) suggests that the phosphate ion concentration in a saline soil solution should be greater than in a non-saline soil with similar phosphate carriers. However, if the concept of a continuous range of solid solutions of basic phosphates tending towards the least soluble form as proposed by Eisenberger *et al.* (24) is accepted, it is conceivable that the "salt effect" would tend

to decrease the level of soluble phosphate in the soil. This is predicted because in the soil system, soluble phosphate is continuously renewed by biological activity. At the same time chemical precipitation in increasingly insoluble calcium forms tends to decrease the level of phosphate in the soil solution. Moreno et al. (73, 74) have shown that the adjustments to the more basic forms is extremely slow under normal soil conditions and compounds of intermediate solubility can be studied by equilibrium procedures. The rate of conversion to the more basic phosphate compounds should be higher in saline soils because of higher average moisture levels and because the degree of dissociation of the intermediary compounds would be increased by the salt effect.

This hypothesis would help to explain some of the contradictions appearing in the literature concerning the effect of soluble salts on the solubility of soil phosphate, since the effect of the salts would depend on the relative composition of the basic phosphates in the soil.

The results presented in Table 7 concerning the quantities of phosphate extracted from saline and non-saline soils by a solution of ammonium fluoride-hydrochloric acid and the lack of correlation between salinity level and sodium bicarbonate extractable phosphate, as shown in Table 4, fail to confirm this hypothesis. However, this evidence is inconclusive because the chemical nature of the phosphate compounds

extracted by these two solvents is not known and because the validity of these solvents as extractants of plant available phosphate is not well established under Manitoba conditions.

Even if this hypothesis is correct, it cannot account for the results of the absorption experiments presented in Tables 6 and 11. These show that, in the early stages of growth, the absorption of fertilizer phosphate increases with increasing salinity to a level of approximately 4 millimhos and then decreases at higher salt levels. These results suggest that the salts either affect the availability of the fertilizer per se or influence the plants ability to absorb fertilizer phosphate.

In order to evaluate the second hypothesis it is necessary to consider the method of application of the fertilizer phosphate. In all of these experiments the fertilizer was applied in granular form, and fed directly into the seed-opener with the seed.

The studies conducted by Lindsay and Stephenson (59, 60, 61) and Lehr et al. (57) and Brown and Lehr (12) indicate that under calcimorphic soil conditions a highly concentrated ring of soluble phosphate forms around a fertilizer granule. This ring rapidly expands as water migrates to the area of high osmotic pressure and as the phosphate diffuses outwards it is precipitated as dicalcium or more basic calcium phosphate. These reports indicate that this is a relatively rapid reaction and the soluble phosphate concentration rapidly decreases to a level comparable to the original soil.

The above authors studied the reactions of monocalcium phosphate fertilizer granules. Bouldin and Sample (5) have conducted similar experiments with monoammonium and diammonium phosphate granules. The results have been similar although the reaction products were not identified. Their results indicated that, in calcareous soil, the reaction products from granules of diammonium phosphate were less soluble than the products from monocalcium phosphate and monoammonium phosphate was intermediate in the solubility of the soil - fertilizer reaction products. Bouldin and Sample (5) also reported that oats were unable to absorb any phosphate from diammonium phosphate granules after a four week, pre-seeding incubation period.

It is believed that the results obtained by Bouldin and Sample (5) with individual fertilizer granules would apply to fertilizer band applications, as practised in Manitoba. The rates of application generally used in Manitoba result in inter-granule distances comparable to the maximum distances of radial movement of fertilizer phosphate measured by these authors. Therefore, the granules would react independently, and no overlapping of their spheres of influence would be anticipated. In more concentrated bands, overlapping would occur and the fertilizer phosphate would be expected to remain in a soluble form for a much longer period of time.

Due to the "salt effect" it is anticipated that these reactions would be more rapid in a saline than in a non-saline soil, because of the effect of the salts on the dissociation of calcium containing compounds. If this is correct, a hypothesis based on the assumption of increased fertilizer utilization due to the effect of the salts on fertilizer solubility, is not tenable because the amount of fertilizer the plant is able to absorb from the fertilizer band is dependent on the length of time the fertilizer remains in a soluble form. Factors which tend to increase the rate of dissolution and reprecipitation of fertilizer phosphate should decrease the amount absorbed by the plant.

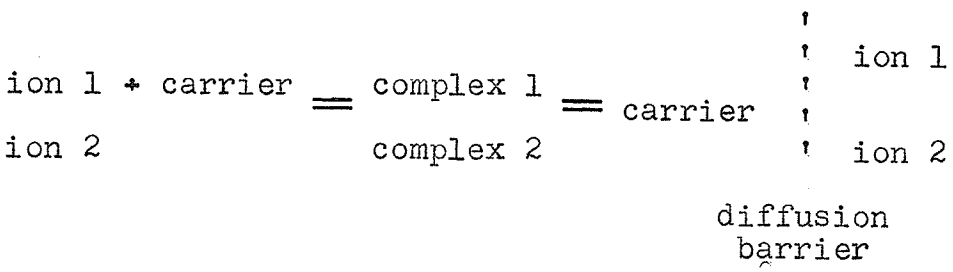
The third mechanism suggested above is worthy of consideration because of the evidence which has been accumulated by Epstein (26) and numerous other authors (15, 28, 38, 46) concerning the inhibitory effects of specific ionic species in the absorption process. These authors postulate that absorption is accomplished by means of metabolicaly produced "carriers" which transport the nutrients across diffusion barriers in the plant. This mechanism can be illustrated as follows:

$$\text{ion} + \text{carrier} = \text{complex} = \text{carrier} \;\Big|_{\text{diffusion barrier}}\; + \text{ion}$$

Studies of absorption based on this hypothesis indicate

that the "carrier" is at least group specific and in some cases possibly specific for an individual ion.

Cases of group specificity have been demonstrated by the phenomenon of competitive inhibition which can be illustrated by the following diagram:



If absorption is limited by the amount of the carrier present in the plant roots the relative concentration of ions 1 and 2 in the substrate will govern the uptake rate when the ions are determined independently, since they are competing for a limited amount of carrier. This type of inhibition has been demonstrated for a large number of ion pairs with similar valencies, (26, 28, 65, 67). Kinetic studies conducted using phosphate as the test ion have shown (46) that phosphate absorption is competitively inhibited by the hydroxyl ion, but other commonly occuring anions such as Cl^-, NO_3^-, SO_4 (46, 54, 67) do not inhibit the absorption of phosphate. This suggests that competitive inhibition of phosphate absorption is not a factor in saline soils when compared with soils of similar pH.

The kinetics of this reaction are similar to the kinetics of enzyme reactions (75) and it is possible that what is

known as non-competitive inhibition in enzyme reactions also occurs in the absorption process. This type of inhibition occurs when some foreign material exerts an effect on the enzyme, either blocking the site of substrate attachment or reducing its affinity for the substrate. Under these circumstances the effect of the inhibitor cannot be altered by increasing the substrate concentration. Neiland and Stumpf (75) report that metal ions often act as non-competitive inhibitors in enzyme reactions. If this mechanism was operating in phosphate absorption in saline soils, it would be expected to decrease soil phosphate absorption in proportion to its effect on fertilizer phosphate absorption. None of the first three mechanisms suggested above would individually account for the observed bipartite relationship (fig. 2) between fertilizer phosphate absorption and salinity level. However, the second and third mechanisms could result in a relationship with a maximum if it is assumed that salts tend to increase the solubility of the fertilizer phosphate and also inhibit the plants phosphate absorption mechanism.

This hypothesis fails to account for the results reported in Table 12 and 13. These experiments showed that phosphate absorption is inhibited in saline solutions, if measured by means of intact plants and comparatively long absorption periods, but when root segments and short absorption periods were used, saline solutions failed to inhibit

absorption. This contradiction can only be resolved by assuming that the absorption mechanism is distinct from the process of permanent accumulation.

Steward and Sutcliffe (91) have suggested that ion absorption in meristematic cells and bacteria is by means of binding to metabolicaly reproduced sites in the cytoplasm. It is suggested that in higher plants these ions are transferred to the vacuole as the cell matures. This assumes a two phase absorption mechanism which can operate independently or, as in the case of vacuolated cells, occur almost simultaneously. The metabolicaly produced binding sites of Steward and Sutcliffe (91) are then identical to the carriers proposed by Epstein (26). Recently Handley et al. (39) published results which indicated that all of the sodium adsorption occurring in the non-vacuolated root tips of corn was open to diffusion exchange with the media while vacuolated cells from the same roots were capable of absorbing non-exchangeable sodium.

Loughman (64) while studying the uptake of phosphate by potato tubers observed that the greater part of the inorganic phosphate in the cell is stored in the vacuole and does not participate in steady state metabolism. The results reported in Tables 18 and 19 indicate that the supply of phosphorus contained in the seed is rapidly converted to readily soluble forms. The concentration of soluble phosphate would be very high in young plants because this conversion rate is

high relative to the growth rate. The cells in the tissue of young plants would not be highly vacuolated and a high proportion of this soluble phosphate would necessarily be contained in the cytoplasm. If the postulates of Briggs (9) and Briggs and Robertson (8) and Butler (15) are correct, concerning the ease of diffusion into and out of the cytoplasm, a high loss of plant phosphorus would be anticipated. Particularly if the growth media had a high capacity for absorbing or precipitating phosphate. The site of the diffusion barrier is not universally accepted and Haapla (37) has recently presented evidence that the plasmalemma is more resistant to diffusion than the tonoplast. This directly contradicts the work of a number of authors (8, 9, 39) and it is possible that the diffusivity of these membranes depends on the age or type of tissue.

The only explanation which appears to be consistent with the experimental results and with current chemical and physiological theory, concerns the interrelation among salinity, growth rate, and phosphate absorption. The relation between plant growth and salinity has been studied extensively. At very low salt levels the growth of wheat, oats and barley is stimulated by increasing salinity (Table 9 A) (76). This is attributed to the accumulation of salts in the osmotic volume of the plant tissue, which creates a more favorable osmotic pressure gradient between plant and soil. (The relationship between auxin control of growth and osmotic gradient control

has not been resolved, however, for present purposes the mechanism is immaterial). As the level of salinity in the substrate is increased above an optimum level, growth decreases rapidly. The decreasing phase of the relationship is attributed to a less favorable osmotic pressure gradient according to the "osmotic inhibition" theory and has been shown to be almost linear (33).

Since the relationship between growth and salinity is attributed to osmotic stimulation followed by "osmotic inhibition" the osmotic volume of the tissue would be altered proportionately more than the total volume. The measurement of free space (Table 17) is an indication of this relationship.

In calcimorphic soils, phosphate fertilizer is applied in a granular form, in direct contact with the seed. As pointed out above this results in a short period of very high levels of available phosphate which is rapidly adsorbed or precipitated by the soil (5). Under these conditions the degree of response obtained from the fertilizer is dependent upon the amount of phosphate the plant is able to accumulate and store in a non-diffusable state during this short period of high phosphate availability. The amount of phosphate absorbed is seen (Table 9 C) to be related to salinity in the same way as growth. The phosphate accumulation rate is stimulated (Table 11) to a greater extent than growth by favorable and unfavorable osmotic pressure gradients.

If the concepts concerning nutrient absorption developed by Steward and Sutcliffe (91), Handley et al. (39) and Loughman (64), are considered relative to the above discussion the relation between phosphate response and salinity can be explained by the following hypothesis:

The phosphate contained in the germinating seed is rapidly hydrolysed to labile forms (Table 18 and 19) which participate in cytoplasmic metabolism. In non-vacuolated cells all of the phosphate exists in the metabolic pool with a higher than normal proportion in inorganic form. As the elongation phase of growth develops, inorganic phosphate in excess of immediate metabolic requirements is transferred to the vacuole by active absorption. (It is assumed that phosphate can be removed from the vacuole to meet the requirements of the developing plant, but this redistribution is not fully understood (91)).

The amount of phosphate that could be absorbed in a non-diffusable form would therefore depend on the osmotic volume of the tissue or possibly on the proportion of the cells in the tissue which become fully vacuolated. (Individual cells are known to have greatly different osmotic pressure, from studies with plasmolysing solutions). It is anticipated therefore that phosphate absorption and growth would be related to the osmotic pressure or level of salinity of the substrate in a similar fashion. Since the accumulation of non-diffusable phosphate would be more directly dependent on the

osmotic volume than growth, it is anticipated that phosphate absorption would increase more rapidly than growth, under favorable conditions and decrease more rapidly under unfavorable conditions (Table 11).

Under extreme conditions (high level of salinity and low phosphate supply in the soil) when the extension phase of root growth is severely restricted by an unfavorable osmotic pressure gradient, phosphate losses by diffusion would be expected to be extreme (Table 2, column 6). However even under these conditions the phosphate level would be unlikely to come to diffusion equilibrium with the substrate (as Handley et al. (39) report occurred with sodium in meristematic tissue) because of the relatively large proportion of phosphate participating in steady state metabolism. It is also probable that mitochondria (91) and other cytoplasmic inclusions in addition to the vacuole are capable of accumulating nondiffusable phosphate.

This hypothesis suggests that the methods employed for measuring phosphate uptake with P^{32} would be unsatisfactory because of isotopic exchange. If a large proportion of the cellular phosphate is accessible to the substrate by diffusion then isotopic equilibrium between plant and medium would be rapidly attained. The P^{32} entering the plant by this process could be removed if the roots were washed in concentrated (relative to the root) solutions of tracer free phosphate

following the absorption period. The wash solutions used in the experiments reported in Table 12 were the same concentration as the absorption solutions (10^{-4}, 10^{-5}, and 10^{-6} M with respect to P). The roots were washed three times by dipping them (1 g of root) in 100 ml of these solutions. If a large proportion of the plant phosphate was at isotopic equilibrium with the solution, the washing with 10^{-6} M P would be inadequate, because calculations indicate that the concentration of inorganic phosphate in the root was approximately 10^{-2} M P. The hypothesis suggests that the proportion of plant phosphate free for isotopic exchange with the solution is affected by salinity. Consequently, the phosphate absorption reported in Table 12 is probably the resultant of two fractions, metabolic absorption and isotopic exchange, the proportions of which vary among treatments.

Since the rate of increase of the osmotic volume is presumably dependent on intermediary metabolism, other environmental factors, such as temperature, which affect metabolism would be expected to have a similar effect on phosphate absorption. The loss of phosphate by diffusion has been shown to be dependent on temperature (Table 14). Nielsen *et al.* (77) have recently published data relating phosphate absorption to soil temperature which is consistent with this hypothesis.

In fertilizer experiments conducted on natural saline

soils it has frequently been noted that the influence of phosphate and the visual symptoms of salt damage are most severe during the early stages of plant growth, prior to the development of the secondary root system. This relationship is illustrated in Table 5. The magnitude of the phosphate response and the apparent free space of the plants, (presuming transpiration carried the larger ash content into the tops through the free space, Hymlo (49) was at a maximum at the June 6th sampling date which corresponds to the period of crown formation in the crop. Unfortunately soil temperature and stage of development cannot normally be compared independently in field experiments and conclusions concerning the effect of the stage of development of the plant are unwarranted. Extension of the hypothesis concerning the role of the osmotic volume of the plant root in the accumulation of phosphate, beyond the very early growth stage would be equally unwarranted.

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